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A STUDY OF THE PRECIPITIN AND COMPLEMENT FIXATION REACTIONS.

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It is well known that if red corpuscles are treated with an inactivated, specifically hæmolytic serum, together with a fresh serum containing complement, the corpuscles are dissolved. No hæmolysis is produced if the fresh serum is not added, or if it contains no free complement. Any procedure which removes the free complement will therefore prevent hæmolysis, and, conversely, we can determine whether any given treatment of a fresh serum removes free complement from it by performing the hæmolysis test with it after such treatment. It is clear from this that the occurrence or non-occurrence of hæmolysis may be used as the indicator of the presence or absence of free complement in the fresh serum.

If serum containing free complement is present when a bacterial extract is mixed with the corresponding antibacterial serum, among other changes occuring, some reaction takes place, in the course of which the free complement disappears. The nature of the reaction and what becomes of the complement is not known, but the indicator described above proves conclusively that there is no complement remaining free after the reaction has taken place.

It has been found that this is a general reaction, which according to some observers, occurs whenever complement is present during the union of any specific antigen and antibody, or according to others, during the union of any specific antigen with its corresponding precipitin. There are also a number of other agencies which are not specific, but which lead to the disappearance of complement.

"It can be seen from what has preceded, that for the specific complement deflection phenomenon we require:

1. A hæmolytic complex consisting of (*a*) specifically hæmolytic serum, (*b*) corresponding homologous red blood corpuscles.
2. Fresh serum containing free complement.
3. An antigen.
4. An antibody specific for the antigen.

If 2, 3, and 4 are brought together for an hour and are then added to 1, no hæmolysis occurs as the complement in 2 has been "deflected" during the union of 3 and 4. Knowing that this reaction always occurs, the phenomenon can be used for purposes of diagnosis, either of the antigen, (3), or of the antibody, (4).

The deflection test has been applied to three main subjects:

I. In the diagnosis of syphilis.

II. To supplement the precipitin reaction in the forensic diagnosis of minute traces of blood, and in the differentiation of blood of various species and subspecies.

III. In the diagnosis of bacteria and of bacterial diseases.

In a former paper one of us (Marshall(1)) traced the origin and development of the deflection method. The present article will deal in detail with (1) the technique and its modifications, (2) the interpretation of positive and negative results, (3) the range of application of the method, (4) its diagnostic value, and (5) lipoidal and nonspecific deflections. We will also describe a comparative study of this and the precipitin method.

I. TECHNIQUE FOR THE SERUM DIAGNOSIS OF SYPHILIS.

1. Preparation of Materials.

A. THE HÆMOLYTIC COMPLEX.

a. The hæmolytic serum.—All observers have used the serum of rabbits immunized against the erythrocytes of sheep. Wassermann, Neisser, Bruck, and Schucht(2) recommend using a serum of which from 0.001 to 0.002 cubic centimeters dissolves 1 cubic centimeter of a 5 per cent suspension of sheep's corpuscles when treated with 1 cubic centimeter of a 0.1 dilution of guinea pig serum. Of this he used twice the exact dissolving dose with each cubic centimeter of 5 per cent sheep's corpuscles. Meier(3) and Michaelis and Lesser(4) use and recommend three times the exact dissolving dose of amboceptor.

Wassermann(2) remarks that the limits of the hæmolytic amboceptor must be determined from time to time. The avidity of complement for the amboceptor varies with the particular immune serum. When the serum grows old, it of itself causes deflection and under these circumstances it can only be used by treating the corpuscles with it and then centrifugating and suspending the corpuscles in fresh salt solution before adding complement.

b. The red blood corpuscles.—The authors quoted(2) note that the corpuscles must come from a healthy sheep which has not been bled too frequently. After frequent bleedings, the corpuscles become too fragile for use. The blood must be defibrinated, and the corpuscles washed and centrifugated until entirely free from serum, and a 5 per cent suspension made in 0.85 per cent salt solution.

c. The complement.—The complement, according to the same authors(2), must

be serum obtained from guinea pigs on the day of the experiment; the serum must be free from hæmoglobin and must be centrifugated until perfectly clear.

b. ANTIGEN.

Wassermann(2) uses extracts in salt solution from various organs of infants who have died of congenital syphilis, made by taking 1 gram of organ, grinding thoroughly in a mortar with 4 cubic centimeters of 0.85 per cent salt solution, plus 0.5 per cent carbolic acid, shaking for twenty-four hours, and centrifugating until the fluid is perfectly clear. He uses 1 cubic centimeter of a 0.1 dilution of this extract for each test tube.

Meier(3) employs only extracts of livers from syphilitic fœtuses, as other organs are less active.

Morgenroth and Stertz(5) suggest that the luetic organs be frozen and kept solid until ready for use, and that from these frozen organs one gram be used for each experiment in preparing a fresh extract.

Marie and Levaditi(6) thoroughly desiccate the fœtal organs and make a powder which retains its strength for months, and for each experiment make up a fresh extract with a little of this powder and salt solution.

The extract of Wassermann is fit for use for about six days, although Meier(3) finds that an ordinary aqueous extract retains its strength for four months if protected from heat, light and air, and if not too frequently centrifugated.

C. THE ANTIBODY.

The antibody is usually obtained from the serum of a patient suspected of suffering with the disease. In a large number of cases the cerebro-spinal fluid has been employed as antibody. Meier(3) draws 6 or 8 cubic centimeters of blood and as quickly as possible obtains at least 1.4 cubic centimeters of serum which is inactivated at once. In the case of children, less blood is drawn, also a smaller quantity of spinal fluid is used. He recommends that the serum or spinal fluid be used immediately because of the danger of rapid deterioration upon standing. In establishing the value of the sero-diagnosis of syphilis Wassermann(2) uses the serum of monkeys first inoculated with syphilis and subsequently treated with subcutaneous inoculations of syphilitic material. He always clarifies the serums and extracts at as short an interval as possible before performing the experiment. Meier finds also that the serum rapidly deteriorates and either loses its deflecting power, or, more usually, causes deflection even with extracts of normal organs.

There are various precautions to be observed in regard to both antigen and antibody. Wassermann and his colleagues(2) make the following observations:

1. Many extracts and serums of themselves alone pick up complement, the amount of extract or serum required varying with the individual preparation and in the case of each, varying with age; 0.1 cubic centimeter of a serum may not block when it is fresh, but after standing it will do so.

2. Any extract or serum showing even the slightest trace of cloudiness or precipitation will deflect and is unfit for use until centrifugated.

3. Frequently a serum or extract which is perfectly clear, absorbs complement. This is especially true of those which have stood long before being clarified by centrifugation or before inactivation.

To avoid this non-specific deflection of complement centrifugate and inactivate immediately after obtaining the serums and extracts; preserve carefully under aseptic precautions in completely filled tubes, closed by fusing the glass, and keep at a constant freezing temperature away from light and air; centrifugate as frequently as is necessary; determine in each individual experiment the non-deflecting dose, and never use a large one.

4. Wassermann and Citron(7) have shown that albumoses, glycogen, fatty stuffs, and lecithin block complement in even small doses. The richer a fluid is in colloidal materials, the more it deflects and vice versâ. Extracts of red blood corpuscles deflect most; spinal fluid, least. As a rule, fresh red blood corpuscle extract does not deflect in a dose of 0.5 cubic centimeter; old extracts deflect in doses no larger than 0.1 cubic centimeter. These various effects are distinct from the specific deflection.

Michaelis and Lesser(4) find that occasionally an extract of syphilitic liver is itself hæmolytic when added to corpuscles, amboceptors and complement. Furthermore, a definitely deflecting serum will often suddenly lose its power after standing.

Meier(3) finds that antibody loses strength upon being kept even in completely filled, tightly sealed and blackened tubes in a cold place. Its action also varies with the age and condition of the complement and of the red blood corpuscles. Wassermann(2) notes that upon standing, even a perfectly clear serum or extract gradually develops a cloudiness or even a slight precipitation. It is very necessary to centrifugate both serum and extract until they are perfectly clear before performing the experiment. The slightest trace or precipitation in the serum makes it unavailable for the experiment. It must be remembered, however, that each centrifugation of a serum or extract decreases its strength, it appearing that the active constituents are carried down in the sediment.

2. Preliminary Experiments.

A. COMPLEMENT.

Most observers, including Wassermann(2), Michaelis(4), and Meier(3) recommend the use of 1 cubic centimeter of a 0.1 dilution of guinea-pig serum, as the standard amount of complement for use in each test.

B. VALUE OF HÆMOLYTIC AMBOCEPTOR.

With each amboceptor a test must be made at the outset to determine approximately its value. This value remains fairly constant, but shows gradual and progressive deterioration. Before each experiment make a preliminary test with 0.1 cubic centimeter of guinea-pig serum to determine whether the hæmolytic amboceptor retains its strength.

Meier(3) simplifies this experiment as follows: He heats diluted fresh complement for one hour at 37°, so that the results obtained will be comparable with those in the rest of the experiment; he then prepares three test tubes with fixed amounts of corpuscles and the heated complement, and dilutions of 1.5, 2 and 3 of the amount of the hæmolytic amboceptor which proved satisfactory in the previous experiments. Thus, if in a previous experiment 1: 200 of amboceptor gave a good deflection, the dilutions of hæmolytic amboceptor tried out in the preliminary experiment will be 1: 300, 1: 400, and 1: 600. He examines this preliminary test after fifteen minutes, thirty minutes, one and two hours. In the first tube he expects to find lysis after thirty minutes, in the second in one hour at the latest, in the third it should be almost complete in two hours. After the first two tubes are positive, he estimates the dose of amboceptor which will be required and continues with the deflection experiment without delay.

C. ANTIGEN.

Meier(3) notes that it is very necessary to test the value of the antigen and to employ fresh serum from an unquestionably syphilitic patient in making the test. If the syphilitic serum is not fresh it may deflect with an extract of

normal organs. Wassermann and Plaut(8) note that the aqueous extract undergoes sudden and rapid alterations in deflecting power. This is confirmed by others including Morgenroth and Stertz.

D. FINAL PRECAUTIONS.

In a preliminary test determine that the suspected serum does not cause deflection with normal extract. If there is enough serum available, test its action upon sheep's corpuscles with complement. Human and monkey serum, normal or luetic is often quite strongly hæmolytic for sheep's corpuscles, at times 0.1 cubic centimeter producing hæmolysis of 1.0 cubic centimeter of a 5 per cent suspension.

The test tubes, glassware and other apparatus must be irreproachably clean, the extracts and serums must be collected and preserved under aseptic precautions, and the experiments must be conducted in such a way as to avoid contamination by bacteria, dust, etc.

3. Details of the Actual Experiment.

a. Amounts of materials.—Wassermann, Neisser, Bruck, and Schucht(2) use 0.1 cubic centimeter of each one of the ingredients, bringing the total volume in each test tube up to 5 cubic centimeters with normal salt solution. Meier(3) dilutes his materials so that he needs only to measure out 1 cubic centimeter of each ingredient, making the volume constant with salt solution. He uses two quantities, 0.2 and 0.1 of the antibody and also of the antigen. Michaelis(4) and others also use 0.2 or 0.1 of serum from the suspected case with 0.1 or 0.2 of extract of syphilitic organs. Landsteiner and Stankovic(9) use 10 parts of 0.8 per cent salt solution; 1 part of serum inactivated at 56° for one-half hour; 1 or 2 parts of extract and 1 part of guinea-pig complement. After one hour at 37° they add 1 part of 50 per cent sheep's corpuscles suspension free from serum and previously treated with two dissolving doses of hæmolytic serum, incubate for one hour and a half at 37°.

Morgenroth and Stertz(5) recommend wider variations in the amounts of substances employed, especially of the antigen. They show that the zone within which deflection occurs is narrow, in one experiment cited deflection occurring when 0.001 to 0.0025 of antigen was employed, but failing to occur with greater or less amounts.

In most of the literature, 0.1 cubic centimeter of the extract of antigen was employed in each test tube. Meier(3) finds that this usually suffices when mixed with 0.1 cubic centimeter of the luetic serum, while it produces no deflection with 0.2 cubic centimeter of normal serum. If the extract of antigen is weaker he uses from 0.2 to 0.4 cubic centimeters with 0.1 cubic centimeter to 0.2 cubic centimeter of the luetic serum, always making a control to prove that the same dose of extract produces no deflection with even twice the dose of normal serum.

Meier(3) notes that the antibody varies quantitatively in different cases and the quantitative test is necessary for exact scientific work. In practice, however, it is necessary to use only two dilutions, neither of which is so strong as a control with normal serum need be in order that the normal serum produces deflection. In practice Meier uses a dilution of one in five of antibody, that is 0.2 cubic centimeter for one quantity and for the other one-half of this, viz, one part in ten, or 0.1 cubic centimeter of antibody, but in a few of his cases 0.005 cubic centimeter (=1: 200) of serum, deflected completely when mixed with luetic extract, while even 20 times the amount (0: 1 cubic centimeter) gave complete lysis, i. e., no deflection, when mixed with extract of normal organs.

Michaelis and Lesser(4) also employ about 0.2 cubic centimeter of serum from the patient.

It is generally customary among all authorities to use 1 cubic centimeter of a 0.1 dilution of complement as a standard dose.

The total volume of fluid employed by different authorities varies from 2.5 to 10 cubic centimeters.

b. Time and temperature.—The general advice given by Wassermann with regard to time and temperature is to allow the antigen, antibody and complement to remain in contact for one hour at 37°, subsequently allowing this mixture to act upon the hæmolytic complex for two hours at 37°. Some authorities allow antibody, antigen and complement to remain at room temperature. The result may be observed upon removing the test tubes from the incubator, or they may be placed on ice and the result noted on the following morning.

Meier(3) thinks it is very important to observe closely the experiment in the incubator and not let it run automatically for two hours. As soon as lysis is complete in all of the controls, remove the test tubes from the incubator and put them on ice without waiting for the expiration of two hours. Frequently from three-quarters to one hour suffices, especially if the corpuscles and hæmolytic amboceptor have been previously mixed for one-quarter to one-half an hour. This is a great improvement, and does away to a large extent with the "Nachlösung" or hæmolysis which develops in some test tubes after being removed from the incubator and placed upon ice. It is even better to bind hæmolytic amboceptor to the corpuscles by allowing them to stand in contact for one-half hour, and remove the excess of serum by centrifugation and suspend the laden corpuscles in fresh salt solution.

c. Controls.—The controls which are required in conducting this experiment are numerous. In addition to the preliminary tests mentioned above, each experiment must be accompanied by the following controls.

A parallel series of tests must be made—

1. With the serum under examination and normal extract. In these controls there should be no deflection; if deflection occurs, it is not specific and the experiment must be thrown out.

2. With standard fresh syphilitic serum and the extract of syphilitic liver. In this control there should be definite deflection, otherwise the extract is defective, and the experiment fails.

3. With standard fresh syphilitic serum and extract of normal liver. In this control there should be no deflection. If it occurs either the standard serum is deteriorating or the total quantity of colloids is sufficient to produce a non-specific deflection.

4. With serum which is certainly not syphilitic and the luetic extract. In this control there should be no deflection. If it occurs the experiment is valueless and a fresh luetic extract must be prepared.

5. With the antibody serum and complement alone. This test should give no deflection.

6. With the luetic extract and complement alone. This test should give no deflection.

7. With the red corpuscles, hæmolytic amboceptor and complement, which has been previously heated to 37° for one hour. In this control there should be complete hæmolysis.

8. With blood corpuscles in salt solution alone.

9. With blood corpuscles and hæmolytic amboceptor alone.

10. With blood corpuscles and complement alone.

11. With blood corpuscles and organ extract alone. In these controls there should be no hæmolysis.

Meier(3) remarks that as experience increases, it becomes less necessary to make daily controls of the standard luetic serum with extracts and of serums

without extracts, for fresh serum in 0.2 cubic centimeter quantities never deflects alone, but on the contrary, increases the rapidity of lysis because of the amboceptors for sheep's corpuscles contained in normal and luetic human serum. The only exception to this rule is that the serum from an animal which has just had a hearty meal may cause deflection and under any circumstance this serum is not useful because of its milkiness. All authorities agree upon the necessity of having these various controls.

d. Tables.—The following tables from various authorities illustrate the manner of setting the deflection test for the determination of the antibody content of the suspected serum.

Wassermann, Neisser, Bruck and Schucht(2) give the following summary of one of their experiments:

Immune serum:

Obtained from a monkey inoculated and subsequently immunized with syphilitic material.

Antigen:

1. Extract of organs from an infant with congenital syphilis.
2. Extract of bone marrow of a monkey killed seven weeks after inoculation with syphilis.

Antigen controls:

1. Extract of organs from a non-syphilitic fœtus.
2. Extract of bone marrow of a monkey free from syphilis.

TABLE I.

1	0.1 immune serum.	0.1 extract luetic fœtus.	Fresh guinea pig complement 0.1.	Union for 1 hour at 37°.	0.002 rabbit serum hæmolytic for sheep corpuscles (2 dissolving doses).	1 c. c. of 5 per cent suspension of sheep's blood.	Blocking of hæmolysis.
2	do	0.1 extract luetic monkey.	do	do	do	do	Do.
3	do	0.1 extract normal fœtus.	do	do	do	do	Complete hæmolysis.
4	do	0.1 extract normal monkey.	do	do	do	do	Do.
5	0.1 normal monkey serum.	0.1 extract luetic fœtus.	do	do	do	do	Do.
6	do	0.1 extract luetic monkey.	do	do	do	do	Do.
7	0.1 immune serum.	Salt solution	do	do	do	do	Do.
8	0.1 normal monkey serum.	do	do	do	do	do	Do.
9	Salt solution	0.1 extract luetic fœtus.	do	do	do	do	Do.
10	do	0.1 extract luetic monkey.	do	do	do	do	Do.
11	do	0.1 extract normal fœtus.	do	do	do	do	Do.
12	do	0.1 extract normal monkey.	do	do	do	do	Do.

The other controls which are not tabulated are: (1) the hæmolytic system alone; (2) blood corpuscles in salt solution; (3) blood corpuscles with complement alone; (4) blood corpuscles with hæmolytic amboceptor alone; (5) organ extract with blood corpuscles.

The volume of fluid in each tube is brought up to 5 cubic centimeters with salt solution.

In this table, numbers 1 and 2 are the only tubes showing the real experiment, all of the others, including the five noted below the table, being employed as controls. These two tubes demonstrate that there is specific luetic material in the organ extracts made from the syphilitic fœtus and the syphilitic monkey, and that there are specific luetic antibodies in the serum of the immunized monkey.

Wassermann, Neisser, Bruck and Schucht(2) also use a simple form of table indicating only the combinations which are brought together in the presence of complement serum, together with the final result upon hæmolysis.

TABLE II.

[Wassermann, Neisser, Bruck and Schucht; Table I modified.[1]]

Serum of normal monkey.	Serum of luetic monkey.	Extract of non-luetic child's liver.	Extract of luetic child's liver.	Result.
----------	0.1	----------	0.1	Blocking of hæmolysis.
0.1	----------	----------	0.1	Complete solution.
----------	0.1	----------	----------	Do.
----------	----------	----------	0.1	Do.
----------	0.1	0.1	----------	Do.

Meier(3) uses the following table to indicate whether a suspected serum "A" contains antibodies, which will produce deflection with a syphilitic extract and with no other. In this table the actual experiment is shown in column I, numbers 1 and 2, while the other tests are controls. In the last column is recorded the degree of deflection.

He does not tabulate the hæmolytic system with its controls.

TABLE III.

Serum.	Amount.	I.		II.		III.		End result.
		Luetic extract.	Result.	Extract of normal organs.	Result.	Salt solution.	Result.	
A----------	0.2	0.2	Complete blocking.	0.2	Complete solution.	1.0	Complete solution.	Serum A = ++++ is a syphilitic serum.
Do----------	0.1	0.1	-----do-----	0.2	-----do-----	1.0	-----do-----	
Standard syphilitic serum.	0.2	0.2	-----do-----	0.2	-----do-----	1.0	-----do-----	
Normal serum-----	0.2	0.2	Complete hæmolysis.	0.2	-----do-----	1.0	-----do-----	
----------	------	0.4	----------	------	----------	1.0	-----do-----	
----------	------	------	----------	------	----------	1.0	-----do-----	

A = Serum to be tested.

[1] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906), **55**, 455.

Instead of recording the presence or absence of hæmolysis and its degree, Meier uses two quantities of antibody 0.1 and 0.2 cubic centimeter and follows Citron's scheme of interpreting the result directly in terms of deflection without first noting hæmolysis. The degree of deflection varies according to the combination of results obtained with these two quantities as follows:

Serum.		Designation of strength of reaction.
0.2.	0.1.	
Complete blocking	Complete blocking	+ + + +
Do	Incomplete blocking	+ + +
Do	Lysis	+ +
Incomplete blocking	do	+

4. MISCELLANEOUS.

Mühsam(10) notes that the negative test must be repeated after several days as the complement binding substances are not always constant in amount, and Wassermann(2) states that any specific serum, which of itself precipitates human albumen, is useless for the deflection test. This refers to the serum of monkeys previously immunized with luetic substances.

II. TECHNIQUE OF DEFLECTION TEST IN FORENSIC PRACTICE AND IN THE DIFFERENTIATION OF BLOOD.

The application of the deflection test for this purpose is essentially the same as in the case just considered. The known quantities are usually the hæmolytic complex, the complement serum, and the specific antibody obtained by immunizing a rabbit with human serum or other known material. The unknown quantity is the antigen, usually a blood clot or blood stain.

Neisser and Sachs(11, 12, 13) adopted the following technique in diagnosing blood clot or blood stain. They employ as the hæmolytic complex a 5 per cent suspension of sheep's corpuscles freed from serum, and the serum of a rabbit immunized against ox corpuscles which is always strongly hæmolytic for sheep's corpuscles also. The serum is completely clarified by centrifugation and inactivated by heating to 56° for half an hour.

The antihuman serum should be of such a strength that 0.01 cubic centimeter will deflect when treated with 0.0001 of human serum. In general they recommend of such a serum the use of 0.02 cubic centimeter as a basic amount for future work and further(12) that the amount of human serum used in the test should be small, 0.0001 being a good dose for testing.

In the deflection experiment, they(11) use 1 cubic centimeter of a 5 per cent suspension of serum-free sheep's corpuscles in 0.85 per cent salt solution treated previously with 0.0015 of hæmolytic serum, which is just twice the lytic dose. In other test tubes quantities of human serum varying from 0.01 to 0.000001 are mixed 0.1 of antihuman serum and 0.05 of guinea-pig serum;[2] after standing for an hour these tubes are treated with the sheep's corpuscles previously laden with amboceptor. The mixture is placed in the incubator two hours, transferred to ice, and the results noted in the morning.

[2] In a later report they use 0.1 cubic centimeter of guinea-pig serum.

In testing blood stains in their three forensic cases, they(12) made extracts of the stain with normal salt solution using very small amounts of the solution.

The work of Neisser and Sachs has been repeated and its value tested by Schütze(14), by Bruck(15, 16, 17) and by Bauer(18).

The antisheep serum used by Schütze(14) is of such a strength that 0.0005 to 0.00033 cubic centimeter dissolves 3 cubic centimeters of 5 per cent suspension of sheep's corpuscles when treated with 0.1 cubic centimeter of fresh guinea-pig serum. He(14) immunizes rabbits either by intravenous inoculation of 3 cubic centimeters of human blood repeated three times or by subcutaneous inoculation of 8 or 10 cubic centimeters repeated five times. One week after the last inoculation the blood is drawn from the animal, the serum placed upon ice to clear, after which it is centrifugated and inactivated for one-half hour at 55°.

He makes his various dilutions in such a manner that the required amount of each constituent is contained in the volume of 1 cubic centimeter, the total of volume in each test tube being brought up to 5 cubic centimeters with salt solution.

Bruck(16) immunized rabbits by intravenous inoculations of 2 to 3 cubic centimeters of serum repeated after eight day intervals and the blood was drawn a week later. Of this serum 0.1 cubic centimeter usually precipitated the homologous antigen in a dilution of 0.1 or 0.05. With such a serum he could differentiate between the blood of the European, Chinaman, Arabian, Malay and monkey, by definite quantitative differences obtained in the reaction.

In differentiating between the serums of various human races two inoculations of the rabbits gave such a strength that using 0.1 of the serum with 0.1 of complement, deflection was obtained with 0.001 of the tested serum. Stronger serums gave doubtful results. Bruck emphasizes particularly the importance of using small doses for inoculating and immunizing the rabbits, so that only very low immunity is obtained. Acting upon this idea in a subsequent series of experiments, Bruck(16) immunized rabbits with 0.5 cubic centimeter every five days, giving three doses intravenously and ten days after the last dose withdrew the serum from the animal and tested it for its deflecting power. With this serum he could differentiate blood, pus and semen, from the same individual, using monkeys for obtaining the antigen for inoculation. In this test he used 0.1 of guinea-pig serum, 0.1 cubic centimeter of sheep's corpuscles plus two dissolving doses of hæmolytic serum, 0.1 cubic centimeter of antigen and a weak immune serum in dilutions varying from one-twenty-fifth to one-four hundredth.

The controls which are used vary according to the purposes for which the test is performed.

The general plan of the experiments of Neisser and Sachs, Bruck and Schütze with the controls and results are shown in the appended tables.

The following table, No. IV, of Neisser and Sachs(11) shows that the deflection is produced by human serum and that the reaction is specific, being given by no other serum except slightly by that of monkeys.

0.1 cubic centimeter of antihuman-rabbit serum+0.05 cubic centimeter guinea pig-serum+varying amounts of various normal serums as possible antigens+ normal salt solution up to 1 cubic centimeter are brought together. After stand-

ing 1 hour at room temperature the mixtures are added to the hæmolytic complex = (0.0015 antiox-rabbit + 1 cubic centimeter 5 per cent sheep's red blood corpuscle suspension).

TABLE IV.

Amount of serum.	Hæmolysis produced with serum from—							
	Man.	Monkey.	Rat.	Pig.	Goat.	Rabbit.	Ox.	Horse.
.01	0	0	Complete	Complete	Complete	Complete	Complete	Complete
.001	0	0	do	do	do	do	do	Do.
.0001	0	Moderate	do	do	do	do	do	Do.
.00001	Trace	Complete	do	do	do	do	do	Do.
.000001	Complete	do	do	do	do	do	do	Do.
0.0	do	do	do	do	do	do	do	Do.

In this table the presence or absence of hæmolysis is noted. It must be remembered that deflection is indicated by the absence of hæmolysis, so that the first three or four tubes with human serum, and the first two with monkey serum are the only ones in which deflection has taken place.

The table of Schütze, No. V(14) shows deflection with human serum. The sheep's corpuscles and hæmolytic serum are mixed separately and not added until the other materials have been mixed and incubated for one hour at 37°.

TABLE V.

[Each ingredient = 1.0 cubic centimeter, total volume 5 cubic centimeters.]

Human serum.	Inactive anti-human serum.	Guinea-pig serum complement.	Antisheep serum.	Sheep corpuscles, 5 per cent.	Result.
.01	0.1	0.1	.002	5	Complete blocking of hæmolysis.
.001	0.1	0.1	.002	5	Do.
.0001	0.1	0.1	.002	5	Do.
.00001	0.1	0.1	.002	5	Appreciable blocking of hæmolysis.
.000001	0.1	0.1	.002	5	Hæmolysis.
.01+1 cc. salt sol.		0.1	.002	5	Complete hæmolysis.
0.0	0.1+1 cc. salt sol.	0.1	.002	5	Do.
0.0	0	0.1+2 cc. salt sol.	.002	5	Do.
0.0	0	0	.002+3 cc. salt sol.	5	No hæmolysis.
0.0	0	0	0	5+4 cc. salt sol.	Do.

TABLE VI.—*Comparison between fresh pig's serum and pig's blood dried on linen about three months.*

Pig's serum, or pig's blood spot.	Anti-pig serum.	Guinea-pig complement.	Anti-sheep serum.	Sheep corpuscles, 5 per cent.	Lysis.
Serum .01	0.1	0.1	.002	5	0
Serum .001	0.1	0.1	.002	5	0
Serum .0001	0.1	0.1	.002	5	0
Extract .01	0.1	0.1	.002	5	0
Extract .001	0.1	0.1	.002	5	Lysis.
Extract .0001	0.1	0.1	.002	5	Lysis.
Serum .01	0	0.1	.002	5	Complete.
Extract .01	0	0.1	.002	5	Complete.
	0.1	0.1	.002	5	Complete.
	0	0.1	.002	5	Complete.

Neisser and Sachs(13) and Schütze(14) call attention to the fact that the very sensitiveness of the test lays it open to error, and in testing for the specificity of a drop of blood on a piece of cloth, error may arise from the fact that the cloth may be contaminated by sweat or nasal secretion, or some other organic body substance. They therefore use as one control a test made with extracts from the cloth adjacent to the blood clot but free from blood.

Neisser and Sachs also used a control with boiled extract, as boiling removes the specific action of the serum deflection.

All tests in which there is evidence of bacterial action must be discarded(14).

Schütze(14) (Table VI) dried pig's blood on linen for three months and made a comparative test of this material with fresh pig serum with the deflection technique. One drop dried on a piece of cloth about 2 cubic centimeters in diameter dissolved in 2 cubic centimeters of salt solution and the extract cleared and filtered, had a deflecting strength of 0.01, while the fresh serum had a deflecting strength of 0.0001, the reaction being specific.

It is seen that there is an abundance of experiments which prove that when carefully performed, the deflection test is just as reliable as the precipitin test in differentiating between blood from different species, and that Neisser and Sachs, Schütze and Bruck, have found it possible to use it where the precipitin test is not available.

Neisser and Sachs recommend the adoption of the deflection test in forensic procedure, as a supplement to the precipitin reaction. They point out that it has certain advantages over the precipitin test which are: That it acts as a control for the precipitin method; that hæmolysis is a much more definite index than minute precipitation; that an opalescent serum is available for use; that it is not necessary to have such high potency serum as is needed in the precipitin test and that it is not necessary to wait for the clearing of the serum, which is so tedious in the older test.

They recommend in every forensic case in which the diagnosis of blood must be undertaken that the precipitin test be performed and upon its completion fresh guinea-pig serum (1.0 cubic centimeter of 1: 10 dilution) be added to each tube and the same materials be employed in making a deflection test.

Schütze agrees with Neisser and Sachs as to the value of the deflection test, while Bruck goes a step further. He recommends that the precipitin test be followed by a deflection test in which *moderately strong* immune serum is em-

ployed in order to obtain a racial differentiation, and thereafter a second deflection test be performed with *weak immune* serum for more delicate differentiation between the various body tissues or secretions.

INTERPRETATION OF RESULTS.

Clear cut results with satisfactory controls are received as conclusive by almost all observers. However, a large measure of confusion has been caused by the fact that some one or more controls are often omitted or because the resulting deflection is not as absolute as could be desired. When Wassermann first put forward this method he advanced the opinion that a positive result could be obtained only in the event of antibody and antigen uniting. The greater amount of work of this nature has been done in the investigation of suspected syphilitic serum, and up to the present time but few cases have been reported in which a positive result was obtained from a patient unquestionably free from infection. These few exceptions will be discussed later together with the change in the interpretation of the reaction which has been brought about by the rapid accumulation of fresh data. It may be stated that practically all investigators agree that the serum diagnosis proves more quickly and more certainly than any other method whether syphilis is or has been present. However, there are a number of other causes which produce deflection of a non-specific character. Bruck(15) reports that serum can be modified in its action by heat. Treatment at 55 degrees for half an hour usually reduces the power so that it is rare to obtain deflection with less than 0.3 cubic centimeter of such a serum. Normal monkey serum when heated for half an hour to 60 degrees acquires strong powers of deflection so that 0.1 will bind four times the dissolving dose of complement twice in succession, or in a single quantitative experiment will bind ten times the dissolving dose. Bruck compares this with the Pfeiffer-Friedberger reaction within the normal body. He finds that monkey serum is affected by heat in this manner with great regularity, guinea-pig serum less frequently, and rabbit serum still less. If the temperature is raised to 65° within 15 minutes the deflecting power is completely destroyed. He thinks it probable that the changes in deflection are due to molecular changes comparable to those occurring on long standing.

In the serum diagnosis of syphilis it has been frequently observed that a specific luetic serum will react with extracts of normal organs, particularly if a large amount of extract is employed. Even in this case, however, it is always necessary to employ a luetic serum in the test. Wassermann(19) was at first inclined to believe that this was a peculiarity of old extracts, but this does not seem to be the case.

A positive deflection may sometimes be observed in the control with an extract of liver itself, and Michaelis(4) also found that normal, non-deflecting serums may become deflecting after being kept frozen for a long time.

Bruck(17) found that his strong immune serum alone would cause deflection in the amount of 0.05 cubic centimeters, and Uhlenhuth(20) gives us a long list of substances of diverse nature which will produce non-specific deflection. Seligmann(21) concluded that absorption of complement is brought about by altering the molecular condition of the colloids in solution, even without causing precipitation. He states that his experiments do not explain the specific immunity reaction, but serve to show that there are other non-specific reactions of a similar nature.

Landsteiner(22) noted deflection when the syphilitic serum was used with extracts from the organs of normal animals, for instance, guinea-pigs' livers, or with alcoholic extracts of organs. We will describe below in more detail the nature of this process as well as the deflection with sodium oleate and lecithin.

However, the positive reactions which are not specific are of such a nature that they do not prevent a careful worker from employing the deflection method with a degree of certainty that is at least comparable to that attaching to many generally accepted clinical methods.

Although a positive fixation reaction with suitable controls give us certain evidence of the existence of syphilis, a negative reaction does not give the same proof of its non-existence. There are various possible sources of error which must be borne in mind in the interpretation of negative results.

According to Michaelis and others, the syphilitic extract of liver is occasionally hæmolytic itself. This function may more than compensate for any possible deflection; aqueous or alcoholic extracts of the liver are very unstable and it is not infrequent that a preparation which has proved satisfactory one day is perfectly useless on the next, and almost all extracts deteriorate on standing. There are also other possible causes which may lead to a negative reaction. They may be summarized as follows: (*a*) the patient has no syphilis and has never had it; (*b*) the patient has been completely cured of previous disease; (*c*) the serum is obtained from a patient during a temporary absence of the specific complement-binding substances from the blood of the patient; (*d*) there are certain refractory individuals whose serum always fails to deflect; (*e*) under energetic mercurial treatment it occasionally happens that the deflecting power disappears. It has also been observed that the reaction is less constant in the early stages and increases in regularity with the age of the disease.

The preceding discussion shows that the particular value of the deflection methods in clinical medicine at present is in the diagnosis of syphilitic diseases.

As Wassermann remarked(19) in December, 1907: "It is, up to the present time, more valuable than the Widal test was after an equal trial * * *. Probably as with the Widal test, some cases will be found which give a positive reaction, although there is conclusive reason to believe that syphilis is not present, but no such cases have been observed so far." He summarizes about 1,500 cases in which the serum diagnosis of syphilis has been attempted, in the great majority of which results were eminently satisfactory. Citron notes that in the primary stages 90 per cent were positive, in the secondary stage between 98 and 100 per cent, in the late cases where the process is active the reaction is practically universal.

The diagnostic value of the test has already been very great to the clinician, the surgeon and neurologist, and to other specialists, and Proskauer professes to employ it as a routine autopsy procedure.

The former article of Marshall(1) showed that there was considerable discussion over the theoretical interpretation of this reaction. Wassermann's idea that it indicated the union of syphilitic antigen with syphilitic antibody at first found almost universal acceptance.

However, Michaelis(23) very soon pointed out that the differences observed in the action of syphilitic serum toward syphilitic liver extract and toward normal liver extract were quantitative rather than qualitative. Then it was shown, as has been mentioned, that syphilitic serum gave positive reactions with extracts of normal organs, with lecithin and with sodium oleate and when comparative tests were made these substances yielded practically the same percentage of positive results as were obtained with the extracts of syphilitic liver. Hence the idea that the reaction was concerned with syphilitic antigen and antibody had to abandoned. Elias, Neubauer, Porges, and Salomon(24) regard the reaction as a precipitation reaction between colloids, the proteins of syphilitic serum having greater instability and yielding a wider flocculation zone than non-syphilitic serum with certain hydrophilic colloids, such as extracts of organs, lecithin, sodium oleate, and sodium glycocholate.

Although the reaction is not specific in Ehrlich's use of the term, yet almost all observers agree that it has a high degree of clinical specificity.

Weil and Braun(25) report positive findings in pneumonia, typhoid, tuberculosis, diabetes, and malignant growths, but their results have not been confirmed by others. Much and Eichelberg(26) obtained positive reactions in 40 per cent of the scarlet fever patients subjected by them to the test. Seligmann and Klopstock(27), Hoehne(28), and Schliessner(29), however, report only negative results in scarlet fever. Wechselmann and Meier(30) and Eitner(31) obtained positive results in two cases of leprosy.

In contrast to the above exceptions, to which a few others could be added, the general verdict is that Wassermann's sero-diagnosis of syphilis is clinically specific.

We have thus far considered the application of the deflection of complement to the diagnosis of syphilis and to the differentiation of the blood of various species of animals. On treating the extract of bacteria with the serum of an animal immunized against the same bacteria, deflection of complement is likewise produced and the application of this principle bids fair to furnish an important addition to bacteriologic technique for the differentiation of closely related microörganisms.

The attempts of Ballner and Reibmayr(32) to differentiate the capsule bacteria and of Gengou(33) to distinguish between the acid fast bacilli by this method were unsuccessful. Schütze(34) concluded that the method was of no value in the study of cholera-like organisms, whereas Ruffner(35) claimed that it enabled him to differentiate between the strains of El Tor and of true cholera and was hence more delicate than the agglutination test. Leuchs(36) could distinguish between typhoid, paratyphoid and the colon bacillus and Vannod(37) between

the meningococcus and gonococcus. Wollstein(38) obtained results contradictory to those of Vannod(37), but Teague and Torrey(39) were able to confirm Vannod's work and to show that furthermore differences such as Leuchs had found between typhoid and paratyphoid extracts exist between certain strains of gonococci. Kolle and Schatiloff(40) after having obtained negative results on using complement deflection in the study of experimental recurrent spirochætosis in mice and rats, finally obtained serum from a man suffering from the disease and with this were able to differentiate between the different varieties of spirochætæ.

Various attempts have been made to apply the method to the diagnosis of bacterial diseases by testing the serum of the patient against an extract of the bacterium in question. In gonorrhœal rheumatism positive results were obtained in a fair percentage of cases by Müller and Oppenheimer(41), Brück(42), and Meakins(43). Positive findings were also reported in typhoid and paratyphoid fevers by Leuchs(36).

Except in its application to the diagnosis of syphilis, where the interpretation of the reaction has already been discussed, the original view of Bordet and Gengou that the method indicates the union of antigen and antibody has been very generally accepted and it is believed by most investigators that it is an antibody *sui generis* which is concerned here and not the precipitins, agglutinins or bacteriolytic amboceptors.

PRECIPITIN TEST.

The value of the precipitin test has been so thoroughly established by the work of Nuttall, Wassermann, Uhlenhuth and many others, that it is unnecessary to review the literature upon this subject. However, there are a few recent works that may be mentioned briefly.

Carnwath(45) has of late described a modification of the technique by which he is enabled to obtain a positive result with very minute amounts of material. The essentials are that the minute blood stain is dissolved in a very small amount of salt solution; that very small glass tubes about 2 millimeters in diameter and 6 millimeters long are employed for the test; that the specific antiserum is introduced by means of a capillary pipette and upon this the suspected fluid is carefully placed in a separate layer. A positive reaction is shown in a few seconds at the zone of contact of the two fluids as a cloud, which gradually spreads upward. This method is very delicate and enables the observer to secure definite reactions with minute quantities of antiserum and of antigen. Carnwath was also able to apply the Neisser and Sachs method of following the precipitin test with the deflection test with these minute quantities, but he agrees with Uhlenhuth that this is unnecessary.

Uhlenhuth in 1903 concluded that it is better in forensic cases to employ only such an antiserum as would produce a definite clouding within one or two minutes with a 1: 1000 dilution of the suspected material when there is one part of serum to 20 of the dilution.

Michaelis and Dehne(46) find that a specific precipitate is dissolved in the presence of an excess of undiluted homologous antigenetic serum. Dehne finds that this is particularly valuable to supplement the test with very minute quantities of antigen; the homologous serum from the known species being added in case of the positive reaction with the extract of suspected material. He also finds that when a specific antiserum produces clouding with the heterologous serum, the precipitate is dissolved equally well by the addition of homologous or heterologous serum.

Weichardt(46) and Stranon(47) attempt to distinguish between individuals by obtaining a highly immune serum, determining its exact value, dissolving as much as possible of the antibody with the serum to be tested and finally adding serum from the homologous individual to determine how much antibody remains. Loele(48) finds he has satisfactory results with precipitin when the material used for inoculating his rabbits was preserved with two per cent formalin in normal salt solution.

EXPERIMENTAL WORK.

In two cases we were called upon to determine the nature of suspected blood stains upon some clothing of natives accused of murder. In the first case the accused alleged that the stains were chicken blood. There were minute flecks upon the surface of a cheap khaki garment which had not been absorbed into the fiber. The stains were examined about five months after the murder, having been kept meanwhile in a safe at room temperature. Extracts made with distilled water and subsequent addition of 1.7 per cent salt solution were colorless, the sediment having a dark, brown color. The hæmin test, precipitin and fixation tests were all negative, and we concluded that the stains were not due to blood. The fixation and precipitin tests were conducted both with antihuman and antichicken serum.

In the second case there was a stain on a cheap cotton garment, which measured about 1.5 by 1 centimeter. The stain had penetrated the fiber and gave an extract which was positive for hæmin crystals, and which gave excellent precipitin and fixation reactions with antihuman serum. The accused claimed that the blood was from a carabao, but as neither precipitin nor fixation tests could be obtained by using anti-carabao serum with the stain, we made a diagnosis of human blood.

In this latter case the precipitin test was eminently satisfactory and gave an excellent ring of precipitate in varying dilutions of the extract. The fixation test also yielded excellent results.

Since in the Philippine Islands and particularly in Manila four races of men, Malay, Mongolian, Caucasian and Negro are represented, it would be of no small importance medico-legally, if we could distinguish between these bloods by means of biologic tests. Bruck(16) had already claimed that this could be done by means of the deflection method, before we undertook these experiments to determine whether either the deflection method or precipitin test furnished sufficiently reliable results for this purpose.

At the same time a comparison of the two methods was made in the differentiation of the blood of the various species of cows to be found in Manila.

A number of rabbits were immunized, some against Caucasian blood, others against Filipino, monkey (*Cynomolgus philippinensis* Geoff.), chicken, and carabao serums. Of the antiserums obtained, one anticarabao

serum gave precipitation with normal salt and with all mammalian serums, though not with chicken and duck serum; another serum, an anti-Malay (Filipino) serum, gave some reaction with the serum of a rat; otherwise all of the antiserums were specific as is shown in the following table:

TABLE VII.—*Precipitin reactions.*

Dilution of serum from—	Serum of anti-Caucasian rabbit No. 3168.	Serum of anti-Filipino rabbit No. 3167.	Serum of anti-monkey rabbit No. 3195.	Serum of anticarabao rabbit No. 3008.	Serum of anti-chicken rabbit No. 1214.
Caucasian	+	+	+	+	0
Filipino	+	+	+	+	0
Monkey	+	+	+	+	0
Carabao	0	0	0	+	0
Chicken	0	0	0	0	+
Duck	0	0	0	0	+
Dog	0	0	0	+	0
Cat	0	0	0	+	0
Rat	0	0	0	0	0

Pieces of filter paper were moistened with a drop or two each of various serums and were allowed to dry and remain at room temperature for from one to three months. They were then extracted with distilled water and an equal volume of 1.7 per cent salt solution was added to each preparation. These extracts were tested against each of the varieties of precipitin serum and it proved easy to differentiate the albumens in this way. By using an anti-Caucasian and antimonkey serum with varying dilutions of the extracts it was not difficult to distinguish even between monkey and human serum.

The precipitin technique used throughout the experiments was the ring method. A drop of undiluted antiserum was placed in a small tube of from 3 millimeters to 6 millimeters in diameter; upon this a dilution of test material was placed with care to preserve the line of contact between the two fluids. A precipitin reaction became evident very quickly in a ring of precipitum at the junction of the two fluids, exactly resembling the albumen ring in the nitric acid test for albuminous urine. It was found that most accurate readings could be made in from 15 minutes to about one hour, the reaction being less distinct after mixing of the two fluids occurs.

The technique of the deflection method has already been discussed at length.

A specimen of Caucasian blood was obtained from an American and specimens of blood were also obtained from a Negro, Chinese, Japanese, Negrito, Tagalog and monkey. Dilutions of these serums were prepared

and simultaneous tests were conducted by the precipitin and fixation methods. The results were as follows:

Precipitin limits of anti-Caucasian serum No. 3332.

Monkey 1 to 1,000.	Negro[3] 1 to 800.
Negrito 1 to 1,000.	Chinese 1 to 2,000.
Tagalog 1 to 2,000.	Caucasian 1 to 3,000.
Japanese 1 to 3,000.	

Precipitin limits of anti-Filipino serum No. 3167.

Tagalog 1 to 2,400.	Negro 1 to 1,800.
Chinese 1 to 2,400.	Negrito 1 to 1,800.
Japanese 1 to 2,400.	Caucasian 1 to 1,200.
Monkey 1 to 1,000.	

The limits here recorded indicate the greatest dilution of the serum which still gave a trace of reaction with the antiserums and, as it is extremely difficult to determine with certainty the exact position of these limits, it seems to us that the differences obtained can have but little, if any, practical value. However, the anti-Caucasian serum does precipitate Chinese, Japanese, Tagalog, and Caucasian serums at greater dilutions than it does monkey, Negro, and Negrito and likewise the anti-Filipino serum gives precipitation with greater dilutions of Filipino, Chinese, and Japanese serums than with those of the Negro, Negrito, Caucasian, and monkey. This last finding is especially interesting because it is directly contradictory to the rather remarkable conclusion arrived at by Bruck(16) from his deflection experiments. He found that anti-Caucasian serum gave deflection with Caucasian serum 1 to 1,000, Arabian 1 to 900, Chinese 1 to 700, Malay 1 to 500; that anti-Chinese serum showed the same limits with Chinese and Caucasian serums, but required more of the Malay serum; that with anti-Malay serum the same limits were obtained for all three serums. He therefore concludes that the protein of the Caucasian contains all the groups of the Chinese and Malay proteins and in addition certain groups peculiar to itself; that the protein of the Chinese contains all the groups of the Malay and certain other groups not contained in the Malay protein.

In deflection of complement tests conducted with dilutions as close together (600, 700, 800) as is indicated in Bruck's tables, the difference in the degree of hæmolysis in the adjacent tubes is so slight that it becomes very difficult to say where the limit of deflection lies and we believe that Bruck's tables indicating a sharp dividing line between the blocking of hæmolysis and hæmolysis are misleading, although we

[3] As the Negro serum was cloudy, it was subjected to repeated centrifugation during the course of these tests and a not appreciable amount of proteid matter was thus removed, so that the dilution was probably really greater than 1 to 800.

recognize the fact that in all probability his results were so tabulated simply for the sake of clearness in presentation. It would appear to us, therefore, that Bruck was not justified in assuming such a fundamental difference in the biological reaction on the basis of such slight experimental differences, and we are confirmed in this opinion by the fact that our precipitin experiments indicate that the established laws of the biological reaction hold also for very closely related serums. In our hands the deflection test with anti-Caucasian and anti-Filipino serum showed differences between monkey and Negro on the one hand and Caucasian, Filipino, Japanese, and Chinese on the other, although we could not distinguish with certainty between Filipino, Caucasian, and Chinese as Bruck claimed he was able to do. Hence we conclude that neither the deflection of complement method nor the precipitin reaction can be used with safety in medico-legal cases to distinguish between the bloods of different races of men.

A further comparison of the two methods was made with the serum of rabbits immunized to carabao serum, and the results are recorded below:

Precipitin limits with anticarabao serum (average of three determinations).

Carabao, 1 to 3,000.	Chinese cow, 1 to 900.
Native cow, 1 to 900.	American cow, 1 to 650.
Goat, 1 to 150.	

The precipitin tests indicate that the native Filipino and Chinese cow are equally removed from the carabao, the American cow being further removed than either of these. The deflection tests gave less clearly defined results than the precipitin reaction and showed the American and Chinese cows to be about equally removed from the carabao, the native being still further removed. It has been shown repeatedly that the antibody concerned in the deflection of complement is not identical with the precipitins, but nevertheless the lack of agreement in the instance cited is rather striking and indicates that the real nature of the deflection reaction has not yet been fathomed.

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TUBERCULO-TOXOIDIN AND IMMUNIZATION SERUM.[1]

By T. Ishigami.[2]

One of the great misfortunes of mankind is that as yet there is no perfect scientific method of successfully combating man's stubborn enemy, tuberculosis. In my belief, the only rational and promising cure for this disease in modern thereapy is the bacteriologic one.

As Koch's preparations prove efficacious on incipient tuberculous patients in many instances only when administered with a careful avoidance of the reaction, the first and most natural step to be taken in our study of the cure is one toward methods of obviating the reaction. After continuous investigations for more than ten years, I have succeeded in preparing two remedial agents of comparatively great efficacy and free from any detrimental reaction.

(1) The first is a chemical preparation from tubercle bacilli and is applicable to incipient and feverless patients.

(2) The other is an immune serum and is applicable chiefly to patients in an advanced stage of the disease.

I introduced these in the belief, based on my own experience of several years, that they were harmless and effective, although not absolutely infallible remedies for tuberculosis. I have since received the corroboration of many practitioners who recognize their efficacy and harmlessness. In this paper I will attempt to describe them briefly.

TUBERCULO-TOXOIDIN.

This preparation is made by chemically dissolving the tubercle bacilli and modifying their toxic property, thus eliminating the reaction which is the common detriment of all other preparations made from tubercle bacilli.

According to the modern theory of immunization, a strong immunity can not be attained without employing strong toxin. Therefore, the question will naturally suggest itself as to whether immunization can be imparted by employing a chemically transformed and harmless toxin. My honored masters, Professors Kitasato and Behring, succeeded in

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, Manila, P. I., February 28, 1908.

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achieving their epoch-making discovery of the serum therapy of tetanus and diphtheria by first attenuating the virus by means of chemical reagents and then immunizing animals with it. Ehrlich's tetanus-toxoid, which is obtained by chemically treating the virulent toxin until it is harmless to animals, still retains the power to immunize them and to neutralize the anti-toxin.

Considering these facts, it is quite a natural step to apply the same principle to the subject of tuberculosis and to expect a successful solution of the problem. From my own experience of many years, I find that for the purpose of curing tuberculosis, bacterial immunization is necessary and that, as the absorption of the tubercle bacilli from the subcutaneous tissue of man and animals is extremely difficult, they must first be chemically dissolved and thus made absorbable.

METHODS OF PREPARING TUBERCULO-TOXOIDIN.

The culture of the tubercle bacilli is well soaked and washed with water to remove the soluble toxin. It is then thoroughly dried and weighed, and, after washing again with water, it is treated with strong sulphuric acid in order to disintegrate the bacterial body and thus extract the inner-toxin and change its toxicity. Then, after adding a large amount of water, stirring and allowing to stand for some time, the fats and aromatic oil rise to the surface, leaving the active substance in the bottom in the form of a precipitate. This precipitate is gathered on a filter paper and well washed with distilled water until it becomes neutral. Five-tenths gram of the dried product is dissolved in 100 cubic centimeters of a solution of weak alkali to form a brown, clear liquid.

Although the preparation of tuberculo-toxoidin is such a simple matter, the duration of soaking in the sulphuric acid must be carefully regulated according to the virulence of the bacilli; otherwise the toxicity may still remain too great, or the whole may be rendered useless by carbonization. Therefore, more or less skill in manipulation is required in preparing the toxoidin.

This substance when injected subcutaneously in man or animals is easily absorbed without local irritation, and, as the toxic property is already changed, comparatively large doses can be injected without harm; yet while it is harmless, it is as effective in immunizing man and animals as Ehrlich's so-called tetanus-toxoid. Hence the name "tuberculo-toxoidin."

The following experiments on animals demonstrate the efficacy and harmlessness of the preparation:

(1) *Test of toxicity.*—No reaction was obtained by the injection of 10.0 cubic centimeters of the tuberculo-toxoidin into the peritoneal cavity of a tuberculous guinea pig, which would have succumbed in twenty-four hours to an injection of 0.1 cubic centimeter of Koch's old tuberculin.

(2) *Prophylactic test.*—Guinea pigs injected subcutaneously with 1.0 cubic centimeter of the toxoidin are generally found to be immune against the inoculation of tubercle bacilli from the fourth until the fourteenth day after the operation.

(3) *Therapeutic experiments.*—If the treatment of a guinea pig subcutaneously inoculated with tubercle bacilli commences within one week of the inoculation, and 0.5 to 1.0 cubic centimeter of the toxoidin is injected subcutaneously about ten times, the disease will either be cured or prevented from making further progress. If, two or three weeks after the inoculation of bacilli, the injection of the above doses is made into an animal with greatly swollen glands, the swelling subsides, the body weight increases, and the fatal period is postponed; whereas a control animal dies in three months, the test animal receiving injection treatment lives over a year. When such an animal is killed, autopsy demonstrates that the tuberculous lesions of the organs are not entirely healed. This is due to the fact that guinea pigs are too susceptible to tubercle bacilli to allow of a complete cure.

A noteworthy fact is that in the guinea pigs treated with the toxoidin, the visceral tubercles generally show a tendency to heal and the number of cells containing bacilli is much greater than in those which have not been thus treated. Moreover, the bacilli in the cells are small and short, evidently representing a degenerate form.

CLINICAL APPLICATION.

From my own experience and the reports of other practitioners who have tried the preparation, the following conclusions may be drawn:

(1) By injecting the preparation in a gradually increasing dose into tuberculous patients without fever, almost every one of them increases in body weight and vital capacity, and becomes conscious of the alleviation of the symptoms.

(2) The bacilli in the sputum are gradually broken up and agglutinated and finally disappear, although in some rare cases small amount of expectoration containing bacilli are found for a long time.

(3) The quantity of opsonin in the patients' blood is found gradually to increase by the injection treatment.

(4) The incipient and feverless tuberculous patients, almost without exception, can be completely cured from within three to six months by injection of this preparation.

(5) In patients in a more or less advanced stage, if nutrition is good, similar results can be obtained. In feverish patients, a satisfactory result is often obtained by means of the injection used side by side with antipyretics. In more serious cases, beyond a certain degree, the treatment is quite useless.

(6) Those patients who are once cured or alleviated by this treatment only very seldom suffer from a second attack.

(7) Out of a total of 772 tuberculous patients, each of whom had received more than fifteen injections of tuberculo-toxoidin in my clinic within the past few years, there were 274 who were completely cured and 258 who were partially cured. These last two figures added together make 532, being 68.91 per cent of the total number of patients. Those who for various reasons discontinued the treatment numbered 107; those who died numbered 29; the remainder, 104.

(8) Out of a total of 778 patients treated with the tuberculo-toxoidin (injected

more than fifteen times) by other practitioners, there were 232 who were completely cured and 228 who were partially cured. These last two figures combined make 460, equal to 59.13 per cent of the total number of patients. Those who discontinued the treatment for various reasons numbered 162; the deaths 63; and the remainder, 93.

IMMUNE SERUM.

The results of previous investigators on the problem of the serum therapy of tuberculosis, although undoubtedly very valuable, have not yet reached a stage to permit of the general application of serum therapy to patients. My own investigations of previous years have also failed, because of the difficulty in immunizing animals against tuberculosis and of the characteristic detrimental reaction of the animal serum upon tuberculous patients. I have finally succeeded, however, by means of the injection of the tuberculo-toxoidin, in preparing an immune serum of a comparatively strong efficacy. I have also succeeded in removing the characteristic reaction of animal serum upon tuberculous patients in the manner mentioned below.

When an animal serum is injected subcutaneously into tuberculous patients, there are often noticed characteristic violent reactions such as acute urticaria about the injected area, redness of the face, palpitation of the heart, increased respiration, itching of the entire surface of the skin and, though rarely, pain in the joints. All these symptoms, which disappear in from five to thirty minutes, are doubtless due, as maintained by Dr. S. Ogata, to the agglutination of the red blood corpuscles.

When the serum of a goat, a cow or a horse is treated with from 2 to 3 per cent of sodium chloride, kept at 50° C. for thirty minutes, and then filtered through a Chamberland filter, it can be clearly shown under the microscope to have entirely lost the power of agglutinating the blood of tuberculous patients or of healthy people, and, usually it no longer causes any reaction, either local or general, on injection into a patient.

EXPERIMENTS ON ANIMALS.

When 0.1 cubic centimeter of Koch's old tuberculin, which is the fatal dose to a tuberculous guinea pig, is mixed with 0.025 cubic centimeter of the immune serum, and the mixture after ten minutes' standing is injected subcutaneously into a tuberculous guinea pig, there is not the slightest disturbance noticed in the animal.

When the phagocytic phenomena are examined according to Dr. Wright's method, my immunization serum presents decidedly more marked phagocytic activity than other sera.

When 0.5 cubic centimeter of the immune serum diluted to four times its volume is injected subcutaneously every other day into a tuberculous guinea pig with markedly swollen lymphatic glands, the swelling of the glands is greatly reduced after about ten injections. By further continuing this treatment, the course of the disease is arrested in spite of the fact that the tuberculous lesions of the organs are not yet completed healed. The microscopic sections show the bacilli engulfed in the cells becoming smaller and smaller, thus indicating the degeneration produced by the serum.

CLINICAL APPLICATION.

Generally speaking, as has already been stated above, my immune serum does not when injected subcutaneously cause any local or general reaction; still, in some exceptional cases of idiosyncrasy, a reaction may be noticed. When, however, the immune serum is administered internally as described elsewhere, it produces nearly the same results as by subcutaneous injection, but without any reaction. Hence, except for cases demanding quick or local results, it will be found safer and more convenient to administer it internally.

The following cases require subcutaneous injection:

(1) The cases of acute tubercular cerebral meningitis in which the exudation is not yet marked.

I have three records of satisfactory cures attained by injecting the serum into children who had fallen into stupor from tubercular cerebral meningitis. I have also several records of much alleviation by the serum injection of cases of cerebral meningitis appearing in the course of pulmonary tuberculosis.

(2) The cases of tubercular peritonitis having painful indurations.

(3) The cases of painful tubercular arthritis.

In the following instances, either the injection or the internal administration is employed, as the circumstances demand:

In cases of pulmonary tuberculosis with high fever or with disordered nutrition, when the patients are unfit for the tuberculo-toxoidin treatment, the serum injection is first to be resorted to. When the symptoms are alleviated and the fever disappears and nutrition is restored, the tuberculo-toxoidin is injected in the usual manner.

According to the results of the serum treatment performed in my sanatarium, out of a total of 189 patients, 43 were completely cured and 63 partially cured. These last two figures added together give 106, being 56.08 per cent of the total number of patients. Those who, for various reasons, discontinued the treatment, numbered 37; those who died, 24; and the remainder, 22.

(1) The average number of injections for those who were completely or partially cured was 55 per capita.

(2) The increase and decrease of opsonins were greater during this treatment than in the tuberculo-toxoidin treatment.

(3) The body weight and vital capacity generally increase as a result of the serum treatment.

(4) The phenomena of agglutination, degeneration, and diminution of the bacilli are similar to those of patients under the toxoidin treatment.

Judging from the percentage results summarized in the figures above, the result of the serum treatment appears to be somewhat inferior to that obtained with tuberculo-toxoidin. As, however, the serum is employed generally in the more serious cases, while the tuberculo-toxoidin usually

is injected in less advanced cases, the above figures are not strictly comparable with each other. If the advanced patients are first treated with the serum until the symptoms are alleviated and are then injected with tuberculo-toxoidin, a much better result is obtained.

INTERNAL ADMINISTRATION OF TUBERCULO-TOXOIDIN AND IMMUNE SERUM.

The subcutaneous injection of the tuberculo-toxoidin is, as stated above, the safest and most efficacious of all modern therapeutic methods for the alleviation of tuberculosis. However, as this form of injection always requires proper precautions, there are many patients who are prevented thereby from receiving the treatment. Moreover, sometimes, though rarely, there are encountered patients of constitutional idiosyncrasy in whom the injection of the serum causes a reaction. For such cases we are necessarily obliged to resort to a simpler method of administering these cures.

I have ascertained by experiments on animals that the internal administration of the tuberculo-toxoidin and immune serum is harmless and efficacious. Consequently, I have tried the same method on patients for the past few years and found it comparatively efficacious and free from any reaction.

It is difficult to obtain results by administering the tuberculo-toxoidin and the immune serum in liquid form. If administered in the form of pills, however, they are partially absorbed without change, as is seen from the following facts:

(1) Those patients in whom the injection of tuberculo-toxoidin causes fever are also subject to the rise of temperature by the internal administration of the toxoidin pills in comparatively large doses.

(2) Those patients in whom urticaria is produced by the injection of the serum also develop the same symptoms on administration of the serum pills in comparatively large doses.

When patients in an advanced stage receive the toxoidin injection, I administer the pills at the same time in the following manner:

The serum pills are given first until all the symptoms are sufficiently alleviated. The toxoidin pills are then substituted and, in the meantime, the number of injections is gradually diminished. The administration of the pills is maintained for a long time after stopping the injections in order to prevent the diminution of the immunity attained. This particular method of treatment has proved itself to be most effectual.

In the liquid state, the efficacy of the tuberculo-toxoidin and of the immune serum is uncertain, probably because of changes due to the action of the gastric juice. In the form of pills they seen partially to escape the action of the digestive juices and to be absorbed from the intestinal wall.

A NEW INTESTINAL TREMATODE OF MAN.

(Fascioletta ilocana, gen. nov., sp. nov.)

By Philip E. Garrison.[1]

(*From the Biological Laboratory; Bureau of Science, Manila, P. I.*)

In April, 1907, during routine examination of fæces, at Bilibid Prison, Manila, P. I., an ovum was found about 100 microns long, oval in form with one end more sharply rounded; shell, light brown in color and of medium thickness, with an operculum at the sharper end; contents rather refractile, colorless, and composed of a mass of yolk-cells, among which the germ-cell could in some cases be distinguished.

In May, and again in September of the same year, eggs of the same description were found in the fæces of two other natives prisoners and in April of the present year still two other prisoners showed the same ovum.

One of the first three patients was discharged without treatment, two were treated for other parasites and the stools examined for the worms which were the source of the eggs in question, none being found.

The two cases of April of the present year (prisoners Nos. 6667-D and 6612-D) arrived at the prison during the same week. The first was treated for a rather heavy infection with *Ascaris lumbricoides*. Several of these parasites were passed, but no other worms were found, although upon subsequent examinations the undetermined ova had disappeared from the stools.

Treatment with male-fern was advised in the case of prisoner No. 6612-D upon the supposition that we might be dealing with an intestinal trematode[2] and this treatment was administered by Dr. E. C. Shattuck, resident physician of the prison. The stools passed after treatment were examined by my student assistants, Mr. Ricardo Leynes and Mr. Rosendo

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[2] The use of male-fern in infections with intestinal trematodes was first suggested to us by Dr. Ch. Wardell Stiles in his lectures at The United States Naval Medical School, upon the theoretical ground that a drug which is effective for cestodes might be also for other plathelminthic parasites.

Llamas, of the Philippine Medical School, who found 21 small trematode worms.

Superficial examination of the parasites showed that they were *Fasciolidæ*, but did not belong to any species reported for man, and after study of stained specimens I was unable to ascribe them to any recognized genus of this family. On May 4, 1908, a preliminary report of the parasite was made before the regular monthly meeting of the Manila Medical Society, giving a brief description, but without definitely determining the systematic position of the worms.[3]

The further study of stained and sectioned specimens has furnished the data for the following more complete description.

DESCRIPTION OF TYPE SPECIMENS.

In addition to the diminutive size of the parasites, they are remarkable for the size and prominence of the ventral acetabulum and for the general contour of the body, which is broadest in the region of the acetabulum and tapers posteriorly throughout fully two-thirds its length. In its anterior third, the body appears almost round, but becomes increasingly flattened dorso-ventrally toward the caudal end. A cephalic cone is absent, but the extreme anterior portion of the body (about one-eighth) is more or less distinctly marked off from the remainder by the prominence of the acetabulum itself and the rapid lessening of the transverse and

[3] The following extract is taken from the proceedings of the monthly meeting of the Manila Medical Society of May 4, 1908:

"Doctor P. E. Garrison.—*A new trematode parasite of man.*

"Author's abstract.—Ova found five times in native prisoners at Bilibid during the past year; 21 worms obtained from last case after dose of male-fern. Patient complained of no symptoms; physical examination negative, except a slight anæmia. Hookworms and whipworms also present. *Morphology of parasites:* Small *Trematoda*, of the family *Fasciolidæ*; 4, 5 to 6 millimeters long by about 1 millimeter broad; broadest at junction of anterior and median thirds; skin without spines; acetabulum near and much larger than oral sucker; pharynx globular; œsophagus very short; intestinal caeca unbranched and extend to posterior extremity; male and female genital pores open separately between acetabulum and oral sucker, slightly to left of median line; testicles posterior, median, one directly behind the other, each divided into anterior and posterior lobe by median transverse constriction; ovary anterior to testicles; shell gland between testicles and ovary; uterus moderately developed; vitellogene glands highly developed, extending from plane midway between acetabulum and ovary to posterior extremity, meeting in median line ventrally and encroaching upon median field dorsally after they pass caudad of the testicles; posterior excretory tract divides just behind testicles into two lateral excretory canals. Ova average 107 μ long by 63 μ broad, with prominent operculum at one end, unsegmented at oviposition, develop ciliated embryo, which hatches in about one week. Specific and generic position of parasite not yet difinitely determined, the indications being that it may be necessary to create a new species and perhaps a new genus also. (Specimens and photographs demonstrated.)"

dorso-ventral diameters anterior to the acetabulum, and also by a more or less distinct transverse depression in the ventral surface slightly anterior to the acetabulum. The anterior portion thus marked off appears in some specimens almost like a cephalic appendage, an appearance which is heightened by the fact that after preservation this anterior part of the worm is frequently bent sharply dorsad out of line with the longitudinal axis of the remainder of the body.

The posterior extremity (about one-fifth) of the body also is more or less distinctly differentiated from the remainder by a slight, but rather abrupt shortening of both the transverse and dorso-ventral diameters and also by its darker color, due to the extension of the vitellaria across the median field in this portion of the worm.

The remaining, middle portion of the body, comprising something over three-fifths of its length, contains anteriorly the large and prominent ventral acetabulum, behind which this portion of the body is more or less distinctly marked off into five longitudinal tracts or fields, namely, two lateral fields along each lateral margin, containing the intestinal cæca and the vitellaria, a median field containing anteriorly the coils of the uterus, and posteriorly, the ovary, shell-gland and testicles, and two light colored sub-lateral fields separating the median from the two lateral fields and marking the course of the two main branches of the excretory tracts.

Dimensions.—Fifteen specimens gave the following measurements in millimeters:

Length.	Maximum breadth.	Maximum thickness.	Length.	Maximum breadth.	Maximum thickness.
4.25	1.00	0.70	5.00	1.00	0.80
4.00	0.80	0.60	6.00	1.35	1.00
4.50	1.00	0.70	5.25	1.00	0.80
5.50	1.25	0.80	4.00	1.00	0.60
5.00	1.00	0.75	4.50	0.80	0.60
5.00	1.20	0.80	5.80	1.00	0.70
4.00	0.75	0.50	4.00	0.75	0.50
4.25	1.00	0.70			

Maximum, 6 millimeters long, 1.35 millimeters broad, 1 millimeter thick; minimum, 4 millimeters long, 0.75 millimeter broad, 0.50 millimeter thick; average, 4.74+ millimeters long, 0.99+ millimeter broad, 0.70 millimeter thick.

Cuticle is smooth and without spines.

Pigmentation is slight and evenly distributed; the fresh specimens were of a semi-transparent red-gray color and the testicles, ovary, vitellaria and uterus were distinctly visible under a hand lens.

Ventral acetabulum.—The acetabulum measures from 480 to 520 μ in diameter (about half the maximum transverse diameter of the body) and is situated with its center at just about the junction of the first and second anterior fifths of the body.

Alimentary tracts.—The *oral sucker* is terminal or slightly ventro-subterminal, the greater development of its dorsal side giving the oral opening a more or less marked inclination toward the ventral surface. It measures from 130 to 200 μ transversely and dorso-ventrally by from 75 to 130 μ antero-posteriorly (about one-third the size of acetabulum). From the oral sucker to the pharynx extends a short, rather broad *prepharynx* which in some specimens appears almost obliterated by the close approximation of these two organs, while in others it is considerably extended, its length in different specimens varying from 10 to 63 μ. The *pharynx* is globular and measures from 150 to 190 μ in diameter. The *œsophagus* is very short (50 to 100 μ), its bifurcation occurring just anterior to the plane of the genital pores. The thin walled *intestinal cæca* pass rather sharply outward toward the lateral margins and then follow these margins rather closely to near the posterior extremity of the body, one cæcum sometimes reaching a slightly more posterior position than the other. Before they reach the equator of the body the cæca become bounded laterally, and also to some extent ventrally and dorsally, by the vitellogen glands.

Excretory tracts.—In the posterior fifth of the body the excretory tract is single and dilated into a cavity of considerable size with irregular, ill-defined walls ("excretory bladder"). On reaching the posterior border of the caudal testicle the tract divides into two lateral branches which pass cephalad between the median and lateral fields above noted to a position dorsad of the acetabulum where approaching the median line, they are separated by only a thin septum. (In some sections the two tracts appear actually to join anteriorly.)

Male organs.—The *testicles* occupy the posterior part of the median field of the middle portion of the body and lie one immediately and directly behind the other in the median line. They are bounded posteriorly and laterally by the excretory tracts. Each testicle is more or less distinctly divided by a transverse circular constriction into an anterior and posterior lobe. In some specimens, this constriction is very slight and the testicle appears almost oval, while in others it is well marked and there may be even slight indentations at other parts of the surface, marking off four or five poorly defined lobules. From each tetsicle the *vas deferens* passes forward through the lateral (marginal) fields to a position dorsad of the acetabulum where the two enter the posterior end of the cirrus pouch. The *cirrus pouch* measures from 560 to 608 μ in length by from 240 to 280 μ in breadth and is situated dorso-anterior of the acetabulum, with its axis directed forward and ventrad, and also slightly to the left side. Posteriorly it contains a large *vesicula seminalis* which receives the *vasa deferentia* and which as seen in different specimens appears to be capable of considerable distention. Within the

cirrus pouch the *cirrus* is more or less coiled or looped and provided with a well developed musculature and a glandular envelope (*pars prostatica*). In each of the specimens, the cirrus is extruded through the male genital pore and externally is curved in from one to two spiral turns.

Female organs.—The *ovary* is globular and situated at just about the center of the body length, slightly to the right of the median line. Posterior to the ovary, and filling the space between it and the anterior testicle is a well developed, globular *shell gland.* The *vitellaria* extend anteriorly to or slightly behind the junction of the anterior with the middle third of the body length or to a plane about midway between the proximal borders of the ovary and acetabulum. Passing posteriorly, they conform closely to the lateral margins and extend slightly upon the ventral and dorsal surfaces, so that on cross section they give the form of a crescent, in the convexity of which lie the intestinal cæca. After passing caudad of the testicles the vitellaria quickly spread across the dorsal surface and meet in the median line, at the same time encroaching more gradually upon the ventral surface until, in the extreme posterior portion of the body they meet in the median line ventrally also and completely encircle the body, enclosing the extremities of the cæca and excretory tract. The *transverse vitello-ducts* cross the sub-lateral fields (excretory tracts) at the level of the anterior border of the anterior testicle, almost at right angles to the longitudinal axis of the body and enter the postero-lateral borders of the shell gland. *Laurer's canal* present. *Receptaculum seminis* absent. The coils of the *uterus* are fairly developed, extend on the left side as far back as the shell gland, and fill the median field between the ovary and acetabulum, being bounded laterally and separated from the cæca by the broad excretory tracts. The anterior extremity of the uterus continues into the well developed *vagina* which passes forward dorsad of the acetabulum directed slightly toward the left and opens at the *female genital pore,* separate from and situated just to the left of the male pore, the two pores being about midway between planes passed through the anterior border of the acetabulum and the posterior border of the pharynx respectively and just behind the bifurcating intestinal cæca. The site of the genital pores is marked by the transverse depression in the ventral surface above mentioned as more or less distinctly separating the cephalic extremity from the remainder of the body.

Ova.—The ova are not very numerous. The shell is thin, light brown in color, with an operculum at the smaller end. In the fæces the egg-contents are colorless and composed of a number of ill-defined vitellogen cells among which the ovic cell could, with difficulty, be detected in some eggs. The ova, in fresh fæces, vary considerably in size and also in their relative length and breath, some being shorter and thicker than

others. The careful measurement of fifty ova in the fresh stool gave the following results, in microns:

99.9×59.2	92.5×62.9	99.9×55.5	92.5×66.6
96.2×59.2	103.6×59.2	96.2×59.2	92.5×66.6
99.9×74.	103.6×59.2	99.9×59.2	92.5×53.5
103.6×62.9	103.6×62.9	103.6×62.9	103.6×62.9
114.7×55.5	99.9×59.2	99.9×59.2	98.0×59.2
92.5×55.5	96.2×74.0	103.6×62.9	103. ×60.
99.9×66.6	111.0×62.9	96.2×62.9	103. ×62.9
103.6×62.9	114.7×66.6	92.5×59.2	103.6×53.5
88.8×62.9	99.9×62.9	96.2×62.9	107.3×59.2
107.3×59.2	96.2×59.2	111.0×81.9	96.2×57.4
99.9×55.5	99.9×81.9	99.9×59.2	94.3×53.5
96.2×62.9	92.5×62.9	92.5×59.2	
96.2×59.2	96.2×59.2	92.5×59.2	

Maximum dimensions: Length, 114.7; breadth, 81.9 μ.
Minimum dimensions: Length, 88.8; breadth, 53.5 μ.
Average dimensions: Length, 99.58; breadth, 53.5 μ.[3]

When the stools, were repeatedly sedimented until their fæcal character was destroyed, the ova, in about 10 days, developed ciliated miracidia which, raising the operculum, escaped from the shell and swam free in the water. Attempts were made to infect several varieties of snails and one variety of fish with the free miracidia, but without success.

Upon the basis of the characters above described, it is proposed to establish a new genus and species in the family Fasciolidæ, of which these specimens shall be the type and for which we propose the names *Fasciolletta ilocana.*

FASCIOLETTA gen. nov.

GENERIC DIAGNOSIS.—*Fasciolidæ:* Body small, elongate, broader anteriorly than posteriorly. Acetabulum near and much larger than oral sucker. Intestinal tract with short, broad prepharynx, highly developed pharynx, short œsophagus, and long, unbranched cæca, which pass along lateral margins and extend to near the caudal extremity of worm. Excretory system consists of a posterior median stem which, posterior to the testicles, divides into two laterally placed canals which extend anteriorly, separating the testicles, shell gland, ovary and uterus from the cæca and vitellaria. Genital pores anterior to acetabulum. *Male organs:* Testicles massive and compact situated one directly behind the other, in the median line, in posterior portion of body, both caudad of transverse vitelloduct. Cirrus and cirrus pouch highly developed. *Female organs:* Ovary compact, unbranched, situated slightly to right of median line at about the equator of body. Receptaculum seminis absent, Laurer's canal present. Vitellaria most highly developed, in posterior one-fifth where superficially they spread over the dorsal and ventral surfaces, more or less completely encircling the body. Anterior to the caudal border of the posterior testicle they are confined to the lateral fields, conforming closely to the lateral margins of the body, external to the intestinal cæca, and reach a position considerably cephalad of the ovary.

[3] Measurements made with Zeiss objective D D (9237), correction collar at 20, micrometer ocular No. 3, tube length 145.

Shell gland well developed, situated between ovary and anterior testicle. Uterus coiled in the space bounded laterally by excretory channels, anteriorly by acetabulum and posteriorly by ovary and shell gland. Ova large, operculated, not very numerous, and develop ciliated miracidium after leaving body of host.

HOST OF TYPE SPECIES.—*Homo sapiens:* Habitat; intestine.

TYPE SPECIES.—*Fascioletta ilocana.*

Fascioletta ilocana sp. nov.

SPECIFIC DIAGNOSIS.—*Fascioletta:* Length, 4 to 6 millimeters; maximum breadth, 0.75 to 1.35 millimeters; maximum thickness, 0.50 to 1 millimeter; greatest breadth and thickness a little posterior to the caudal border of the acetabulum. Posteriorly, the body attenuates gradually throughout two-thirds its length to a rounded caudal extremity; anteriorly, for about one-third its length, to a rather sharper cephalic extremity. In the posterior half the body becomes increasingly flattened toward the caudal end. Oral sucker terminal or slightly ventro-subterminal, small (130 to 200 μ transversely by 75 to 130 μ deep); dorsal lip much larger than ventral, giving the sucker a ventral inclination. Ventral acetabulum about three times as large as oral sucker (nearly globular, 480 to 518 μ in diameter), situated with its center at about the junction of the first and second anterior fifths of the body length. Skin smooth and without spines. Pigmentation slight and evenly distributed. Prepharynx from 10 to 63 μ long. Pharynx globular, from 150 to 190 μ in diameter. Œsophagus short (50 to 100 μ). Intestinal bifurcation immediately anterior of plane passed through genital pore. Intestinal cæca thin-walled; follow rather closely the lateral margins of body to near its posterior end, being partly inclosed by the vitellaria throughout considerably more than the posterior half of their course. Genital pores open upon the surface separately to the left of the median line and slightly posterior of a plane midway between posterior border of pharynx and anterior border of acetabulum. *Male organs:* The cirrus pouch is from 560 to 608 μ long by from 240 to 280 μ broad; situated antero-dorsad of the acetabulum, with its longitudinal axis directed antero-ventrally and slightly to the left. Posteriorly it contains a bladder-like vesicula seminalis which receives the vasa deferentia and gives origin to a well developed cirrus which takes a more or less coiled course to the male genital pore through which it may protrude in from one to two spiral turns. Vasa deferentia divergent. The testicles lie one immediately and directly behind the other, occupying the median field just caudad of the transverse vitello-ducts. Each testicle is more or less distinctly divided into an anterior and posterior lobe by a transverse, circular constriction, and other slight indentations of the surface may indicate ill-defined secondary lobules. *Female organs:* Ovary globular, situated at equator of body, slightly to right of median line. Vitellaria highly developed, extending antero-posteriorly from the caudal extremity to a plane midway between proximal borders of ovary and acetabulum. Cephalad of the posterior border of the testicles they lie along the extreme lateral margins, filling the space between margins and cæca and extending somewhat upon the dorsal and ventral surfaces, thus inclosing the cæca dorsally and ventrally between the two superficially placed vitellogen layers. Caudad of the testicles, the vitellaria spread over the dorsal surface and meet in the median line, at the same time gradually encroaching upon the median field ventrally. In the extreme posterior portion of the worm (about one-tenth of its total length) they may meet in the median line ventrally as well as dorsally, thus completely inclosing the caudal extremities of the cæca and excretory tract. At the anterior border of the anterior testicle the transverse vitello-ducts pass inward and slightly forward to the well-developed,

globular shell gland which fills the space between the anterior testicle and the ovary. Uterus fairly well developed; its coils filling the median fields between the excretory tracts from the acetabulum to the ovary on the right side and extending caudad of the ovary on the left to the border of the anterior testicle. Its anterior extremity is continued into a well developed vagina which passes diagonally across the median line dorsad of the acetabulum to reach the female genital pore, which is situated just to the outer side of the male pore.

Ova from 88.8 to 114.7 μ long by from 53.5 to 81.9 μ broad, averaging 99.58 by 56.04 μ; develop miracidium in about 10 days after leaving host. Further development unknown.

HABITAT.—Intestine of man.

TYPE LOCALITY.—ILOCOS SUR, Northern Luzon, Philippine Islands.

TYPE SPECIMEN.—Number 240-A (co-types number 240), Helminthological Collection, Bureau of Science, Manila, P. I.

FREQUENCY, LOCALITY, AND PATHOGENISIS.

Over 5,000 native Filipinos, representing all parts of the Islands, have been examined for intestinal worms during 1907 and the first three months of 1908. That only five infections with *Fascioletta* have been encountered would seem to indicate a very low frequency with regard to the population of the Islands as a whole.

However, all five of the infected prisoners came from the northwestern provinces of Luzon, and two of them, including the one from whom the worms were obtained, had lived all their lives in the Province of Ilocos Sur. Accordingly, there is some indication that the parasite may not be equally distributed throughout the Islands.

Only the two cases which appeared in April of the present year were examined clinically. Questions and answers had to be passed through two interpreters (Tagalog and Ilocano) and only fragmentary and rather uncertain information could be secured. Both prisoners had lived in Ilocos Sur all their lives. They had worked bare legged in fields overflowed with water; fish was an important part of their diet; they had not suffered from any sickness except "fever"—which facts, excepting possibly the last, would apply to the great majority of the population. At the present time neither complained of any illness nor would either admit having or having had any intestinal trouble. Physical examination was negative with the exception that one of the prisoners was somewhat anæmic, the hæmoglobin registering about 85 per cent. In this case infections with hookworms and whipworms also were present.

ILLUSTRATIONS.

ac.	=acetabulum.	L. c.	=Laurer's canal.	t.	=testicles.
cir.	=cirrus.	m.	=mouth.	trs. v.d.	=transverse vitello-duct.
cir. p.	=cirrus pouch.	m. g. p.	=male genital pore.		
exc. p.	=excretory pore.	œs.	=œsophagus.	ut.	=uterus.
exc. tr.	=excretory tract.	ov.	=ovary.	va.	=vagina.
f. g. p.	=female genital pore.	p.	=pharynx.	vd.	=vas deferens.
g. p.	=genital pores.	pp.	=pre-pharynx.	v. g.	=vitellogen glands.
int.	=intestinal cæca.	sg.	=shell gland.	v. s.	=vesicula seminalis.

(Photographs by Charles Martin, Bureau of Science.)

PLATE I.

FIG. 1. Photograph. A group of eight specimens of *Fascioletta ilocana*, natural size.

2. Photomicrograph of type specimen from ventral surface. Stained with carmine and picric acid. Enlargement about 18.

3. Semi-diagrammatic drawing showing anatomy.

4. Photomicrograph of longitudinal section in median line. Stained with carmine and picric acid. Enlargement about 20.

5. Semi-diagrammatic drawing showing apparent structure and relations of cirrus and cirrus pouch.

PLATE II.

FIGS. 6, 7, 8, 9. Semi-diagrammatic drawings of transverse sections through (6) cirrus pouch just caudad of genital pores; (7) ovary; (8) posterior testicle; (9) plane midway between testicle and caudal extremity.

FIG. 10. Photomicrograph of ova of *Facioletta ilocana* and *Trichuris trichiura*.

FIGS. 11, 12, 13. Photomicrographs of ova (11) in fresh fæces; (12) showing developed miracidium; (13) showing operculum lifted after escape of miracidium. Enlargement about 320.


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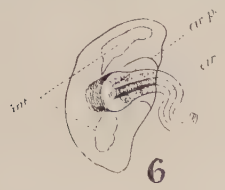
PLATE I.



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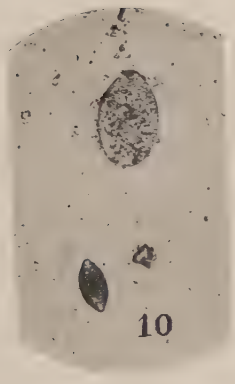

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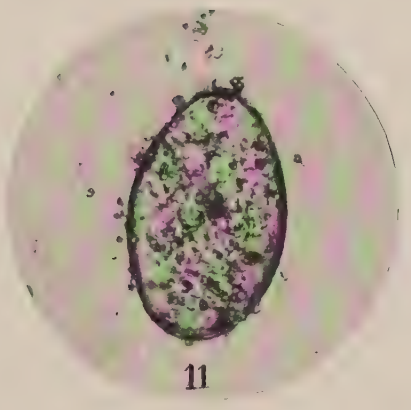

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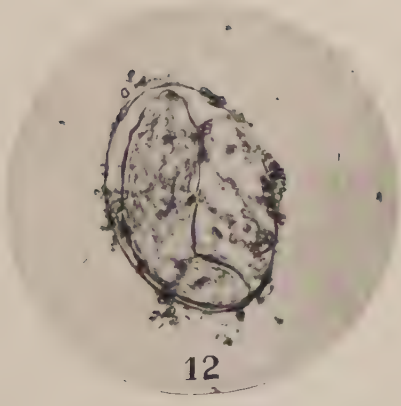

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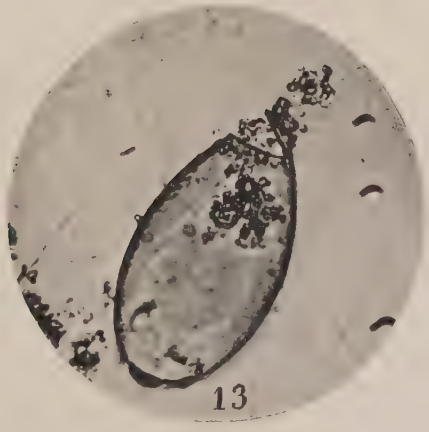

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PLATE II.

BLASTOMYCOSIS OF THE SKIN IN THE PHILIPPINE ISLANDS.[1]

By James M. Phalen and Henry J. Nichols.[2]

I. INTRODUCTION.

Our instructions from the Surgeon-General of the Army state that "parasitic diseases of the skin deserve further study and attempts should be made to isolate and cultivate the fungi causing them." Blastomycosis we have found to be a particularly suitable subject for such a study because of its prevalence and lack of recognition. Blastomycotic infection of the skin was first described in 1894 by Gilchrist and generalized blastomycosis by Busse in the same year. Since then a considerable literature has been produced, in large part by American authors, as is shown by reviews by Hyde and Montgomery, Hektoen and others. The term blastomycosis is a general one and is used here for convenience to designate pathologic conditions produced by double contoured, budding bodies. The botanical relations of these forms to yeasts and fungi, their relations to each other and to such bodies as those of coccidioidal granuloma have not been sufficiently worked out to permit of exact classification. Accordingly, we shall simply call attention to the clinical peculiarities of the disease as we have seen it, to the nature of the organisms and of the cultures obtained and to the presence here of other forms of blastomycosis.

II. PREVALENCE.

We believe that cutaneous blastomycosis is one of the common parasitic skin diseases among both natives and white men in the Philippine Islands. The dispensary clinic of the University Hospital, in Manila, is a rich field for observing these cases among natives. Through the

[1] Read by abstract at the Fifth Annual Meeting of the Philippine Islands Medical Association, Manila, P. I., February 27, 1908.

[2] Captain, Medical Corps, United States Army, and first lieutenant, Medical Corps, United States Army, constituting the United States Army Board for the Study of Tropical Diseases, as they occur in the Philippine Islands.

kindness of Doctors Saleeby and Winsor we have been able to study a number of patients at the Dispensary and usually have been able to find without previous arrangement one or more cases. The physicians in charge say that at least one new case of this form of disease applies for treatment each week. Among 50 natives examined on one morning we found 5 cases. In the last eight months we have seen 7 officers with various degrees of the disease and have ceased to keep track of the exact number of enlisted men affected. The infection has been seen in Mindanao, Jolo, Cebu, Panay, Samar, and Luzon, and there is every reason to believe it to be widespread throughout the group of Islands. This is somewhat surprising because among skin diseases common in the Tropics, no mention is made of this condition by Manson, Scheube, Mense, Macleod in Albutt's System or Jackson, nor is the term found in special articles on the skin diseases of different tropical countries. The only account of this form of infection in the Tropics, which we have found, is by Ashburn and Craig. They had a white patient, age 40, with five lesions of two years' duration on his face, resembling ring-worm. Scrapings stained by the modified Gram method showed round, double-contoured bodies lying in and between the epithelial cells. The patient stated that the disease was common among natives, but rare among whites. He improved under potassium iodide internally. No cultures were obtained. There are several references to blastomycotic ulcerations in the East by Sakurane and Okugawa, Japan, 1905; Lukis, India, 1907; Strong, Philippine Islands, 1906, and Shattuck, Philippine Islands, 1907, but the lesions described seem to be different from those with which we are now concerned.

III. DIFFERENT FORMS.

Clinically the disease appears in three distinct forms, although there are intermediate types from the mildest to the most severe.

A. The mild cases somewhat resemble the commonly observed skin infections with ordinary fungi. The lesions are elevated little, if at all, above the surrounding skin, are irregular in outline, and the surface when freed from scales presents a smooth, reddish surface. The lesions itch considerably, but otherwise give no discomfort. The couse is toward a progressive extension of the patches, with marked induration of the affected skin. The tendency to appear in unusual locations, and the frequency of a symmetrical distribution are the features which distinguish it clinically from ring-worm, with which it has many features in common. It has been observed to occur on the back of the hands, the forearm, shoulder, face, front of the leg, and the toes.

CASE I.—In fig. 1 is given an illustration of a lesion of this class. This patient, a sergeant of an Infantry regiment, first came to the Philippine Islands in 1904 and went to Camp Jossman, Island of Guimaras, for station. In the spring of 1905, while cutting a clump of bamboo at that post, he scratched his

right wrist on a thorn. The cut bled a little and he sucked it. A little later the spot became reddened, and itched greatly, and it gradually spread around the wrist. A similar spot appeared upon the palm of his right hand. These spots were treated with various antiseptic ointments and lotions for nearly two years, without much effect until, while at Mount Gretna encampment in 1906, they were healed by the application of a strong alcoholic solution of mercuric chloride. Shortly after, small spots of a similar character appeared upon the left wrist and left leg, and these have persisted and have continued to spread ever since, more rapidly since his return to the Philippines in July, 1907.

He came to us in March 1908, somewhat anxious about his condition as he had been told by a Spanish physician that the disease was likely to affect his internal organs. The lesions present at that time were situated on the outer side of the left leg near the knee and on the back of the left wrist. There were several areas, each 5 to 8 centimeters in diameter, irregular in outline, slightly raised, with a well-defined edge and covered with scales which, when removed, left a pink, glistening surface.

The surface feels rough and indurated. No crusts nor exudate are present. The scales, when removed, treated with a potassium hydrate solution and examined under a high-power lens, show in abundance the organisms illustrated in figs. 6 and 7, a more detailed description of which will be given later. Attempts to cultivate the organism from this group of cases proved unsuccessful. A picture of a section of the skin is shown in fig. 8.

From a study of the sections, the process appears to be twofold. On the one hand there is an overgrowth and widening of the papillary layers. The papillæ show a definite downward growth and often include a small island of connective tissue which appears somewhat like a miliary abscess, but contains no polynuclear cells. On the other hand, the corium in places infiltrates the epithelium, producing a fibrosis and degeneration of the epithelium so that as the section is moved across the field, one part will show an excess of epithelial elements and the next a loss with an increase of connective tissue. A marked infiltration with round cells appears in the deeper layers of the corium.

The majority of these cases are of many months', or even of years' standing. The mildest yield to strong local antiseptics, others only to potassium iodide internally. These infections are usually passed over as a variety of "dhobie itch" and even on microscopic examination of the scales often nothing is seen unless the oil immersion lens is used.

B. The second type of the disease is the one most frequently encountered. In these cases the lesions are in quite large areas, sharply circumscribed and considerably elevated above the surrounding, healthy skin. They are frequently observed to have a border raised above the rest of the patch, this ridge being beset with "miliary" abscesses covered with crusts. The remainder of the area has a red, smooth surface covered with scales.

Case II.—The patient is a Filipino girl, 8 years of age, from the Tondo district of Manila. No history as to the beginning of or the duration of the disease is obtainable. The lesion shown is irregularly circular in outline, about 7.5 centimeters in diameter, situated back of the left axilla. It presents a

crescentic ridge 3 to 6 millimeters high and 13 to 20 millimeters wide, with a concavity upward extending along the lower edge and sides of the patch. This ridge has a number of small pustules opening upon its surface, and is covered by a crust made up of the dried discharge. The center and the upper part of the affected area is occupied by indurated tissue, with a smooth surface covered by fine scales. A smaller area, 4 by 5 centimeters, of a similar character occupies the outer side of the right elbow. This area has a ridge along the posterior border, with some superficial erosion. The rest of the lesion is made up of indurated tissue of a like character to the larger lesion. The scales and crusts show a great quantity of blastomycetes.

CASE III.—This patient is an officer in a Scout organization, and presents the following history: In the early part of 1904, while stationed at Camp Connell, Samar, he noticed a small papule near the middle of his left cheek. He considered it of no consequence, and so does not remember much of its early characters, except that it itched considerably. He does not recollect any injury or abrasion of the face previously. From this beginning the present lesion has extended, at first downward, then forward and upward; at first it was quite superficial and the earlier places were healed by the local application of nitrate of silver. As is customary in these cases, the eruption recurred almost immediately and the patient has never been free from it since.

When first seen by us, in April, 1908, there was present on the left cheek a large, crescentic patch extending parallel to the lower jaw, presenting an indurated ridge along the convexity of the patch and induration of a less degree inside.

The whole area is red and shining, except where covered with scales. Along the ridge, a number of more indurated spots exist, lighter in color, which upon opening exude a small drop of white pus. There are some crusts covering small cavities also containing pus. Examination of the scales and pus in this case reveals in large numbers the same organisms found in the others.

CASE IV.—C. C., Chinaman, 40 years old, a shopkeeper in the Santa Cruz district of Manila, seen at St. Luke's Dispensary. No history is obtainable, except that the lesions present are of eight months' duration, came on gradually and were still extending. This patient has a circular patch, 5 centimeters in diameter, over the center of the back of his neck. This patch has a raised, indurated border and a central portion, indurate, but apparently healed, on the level with the rest of the skin. The surface of the raised border is smooth, glossy and of a dark red color. This patient has also a round, elevated spot, 12 millimeters in diameter at each angle of the mouth and on the right side of the lower lip a patch extending laterally from the angle of the mouth to the median line, and reaching downward for about 25 millimeters, the lower border being scalloped and raised in a sharp ridge. The lesions are bright red and glossy. The scales show a large number of organisms which are indistinguishable from those found in the other cases; a culture was obtained from this case, which will be described later.

A number of other cases of this class have been observed (fig. 2). They all give a history of a long, chronic course, and of resistance to treatment. They have been diagnosed as syphilis, leprosy, tuberculosis and other diseases. However, with the exception of tuberculosis, there is little resemblance to these infections. The case of cutaneous blastomycosis, reported by Ashburn and Craig in 1906, belongs to this second

class. Both in appearance and in microscopic findings their description coincides with those that we have given.

The histologic picture of type B is intermediate between those of A and C. The papillæ show more marked overgrowth and the upward growth of the connective tissue reaches the surface of the skin and thus accounts for the formation of crusts. There is a greater infiltration in the deeper layers of the corium. The infiltrating cells are almost entirely of connective tissue in various stages of growth, but there are some unusual cells, large and round, which when stained by hematoxylin and eosin, have a deep black nucleus and a rim of pink protoplasm.

C. This form answers clinically quite closely to the descriptions of cases of cutaneous blastomycosis reported from the United States. One well-marked case of this class has been under observation for the past three months, and one other was seen at St. Luke's Dispensary. (Figs. 4 and 5.)

Case V.—B. C., male Filipino, 34 years old, a resident of Pasay, and a coachman by occupation. He was born in Pasay and has lived there all his life. He has been married for twenty years, had five children, two of whom died of smallpox, two of intestinal trouble and one, 11 years old, is alive and well. Ten years ago he acquired a small venereal sore which healed in a week. He has no further symptoms to indicate that the sore was syphilitic. There is no evidence of tubercular infection. Two years ago a sore appeared on the right buttock, near the anus, on the site, the patient thinks, of an abrasion due to horseback riding. This healed after three months of dispensary treatment. One year ago, it reappeared on the right buttock, since which time it has spread gradually to its present dimensions.

Present condition (fig. 3).—The lesions occupy nearly the whole of the right buttock, and extend several inches over on to the left. They reach downward on to the back and inner side of the right thigh and up into the groin in front on the right side. The greater part of this area is occupied by scar tissue, but the process is still active in a ridge 5 centimeters wide along the left border of the patch, in an oval area 5 by 7.5 centimeters in the median line above, in the extreme right edge, in the lower part of the right side and in the right groin. Over these areas the surface is raised 3 to 6 millimeters above the surface of the skin. In places there is a fine, papilliform growth with deep fissures intervening and in others broad, warty surfaces, dry or covered with large scales. The papilliform elevations are frequently covered by a large, yellow crust, and the intervening fissures contain pus loaded with blastomycetes.

The patient was put on ascending doses of potassium iodide, and within a week the papillæ began to contract and became smoothed over and ceased to secrete an exudate. In two months the disease was apparently entirely cured, although some induration remained. A culture was obtained from this case.

A section (fig. 8) from a lesion of this form shows a great overgrowth of epithelium, which superficially resembles an epithelioma. However, the cells are more regular in size, shape, and arrangment. Pearls are occasionally seen. The increase of connective tissue is also very marked and it reaches the surface in wide processes, showing a great deal of exudation of blood cells. The epithelium in its growth incloses a great many small areas of connective tissue. The hair and sweat glands are apparently not involved in the overgrowth.

Clinically it seems as if the milder forms did not develop into the more severe. This can only be decided by further observation, especially of cultures obtained.

The diagnosis depends on finding blastomycetes in the lesions constantly and in such number that they can not be regarded as accidental. Control examinations of scales, crusts and pus from other lesions have shown occasional blastomycetes, but these are not constantly present nor in such numbers as are seen in the lesions described. Of course, sections and cultures are desirable, but in our experience the latter are difficult to obtain and are unnecessary before starting treatment. After seeing a number of cases we have been able to pick out others from their clinical appearance. In the milder forms the presence of blastomycetes and absence of any fungi in a manifestly parasitic disease differentiate the affection from ring-worm, *Tinea imbricata,* etc. The severe forms may be taken for tuberculosis or syphilis. If tuberculous they will not heal up in one to two months of treatment with potassium iodide. The history and clinical appearance differentiate the affection from syphilis, as in the latter disease there is no such overgrowth of the epithelial structures. In tertiary yaws the lesions are ulcerative, while in this form of blastomycosis of the skin no definite ulcerations have been seen.

IV. ORGANISM AND CULTURES.

We have been unable morphologically to distinguish any difference in the organisms from the various cases. If a scale is taken from type A, or a crust or some pus from either B or C and macerated thoroughly in a ten to twenty per cent solution of potassium hydroxide, the organisms become very distinct. Their most striking feature is a double contour, usually less than 1 μ thick, but occasionally some are seen in which it is 2 μ. There is a great variety of forms; the most frequent is the round, measuring 5 to 10 μ; many minute ones of the same kind are seen, 2 to 3 μ in diameter, often in clusters; budding forms are frequent and others making a figure of 8. Another frequent appearance is the rod-like one with rounded ends. Some forms are elongated and in phantastic shapes. These organisms stain with difficulty, a faint rim of blue being often all that can be seen with the modified Gram's stain. No internal structures have been made out, nor is any evidence of sporulation seen except in cultures.

In sections the organisms are found in small numbers in the types A and B, in the more superficial layers of epithelium between the cells. We have found no differential stain for them. With hæmatoxylin and eosin they stain to a light brown and often have a halo about them. They are most readily demonstrated (fig. 10) in unstained sections treated with potassium hydroxide and mounted in glycerin. The double

contour is then fully brought out and this, with buds, makes identification certain. In type C they are found more deeply in the section, but always among the epithelial elements.

Scales from type A failed to yield a culture although when incubated for a week and examined they showed a great increase, with elongation and segmentation of the organisms which approached the cultural forms seen in fig. 12. Scales from type B were washed in water, soaked in absolute alcohol one hour, washed in salt solution and put on plates and slants of maltose and glucose agar. Many remained sterile, a few showed a subtilis-like organism; one on glucose agar, after four days' incubation, gave a filamentous growth in pure culture. This culture on being transplanted grew more readily on sugar media and potato, but showed only a slight filamentous growth on plain and glycerin agar either at room or body temperatures.

The organism grows on potato, at room temperature, in prominent, light brown folds (fig. 10). At body temperature the folds are black. Microscopically (fig. 11) the growth consists of short, blunt branching and segmented processes and in round forms, 20 to 30μ in diameter filled with a number of small, round spores.

The growth on glucose-agar is abundant, forming brown folds in the center and lighter filamentous processes at the edges. No aërial spore-bearing hyphæ are seen. Microscopically (fig. 13) the processes are longer, more slender and no sporulating forms are seen.

The development on maltose-agar is less marked and consists of a brown film on the surface and a white growth penetrating deeply into the medium (fig. 13).

In glucose-bouillon a floculent white ball is found at the bottom of the tube; the fluid above is clear. A slight liquefication occurs in gelatin. Milk is coagulated, but not acidified. No fermentation takes place in lactose, glucose and maltose tubes.

No marked difference in the growth appears at room and body temperatures, except in its color on potato which is noted above.

This organism when injected subcutaneously in guinea pigs, produces a small nodule which soon disappears; double contoured budding bodies are found in the substance of the nodule. On injection into the peritoneum similar nodules are found in the omentum which contain round forms and remains of the filamentous growth injected.

A growth was obtained from type C in the same way, although most of the tubes remained sterile. This growth shows a preponderance of yeast-like forms in the center of the culture with filaments at the edges; the center often retains Gram's stain, while the margin stains brown.

On agar a brownish-black, pasty growth with lighter colored filamentous borders appears along the track of the needle. Growth is not abundant (fig. 14).

Glucose-bouillon gives numerous filamentous islands of growth in the body of the tube with a brown film at the surface. Glucose, lactose and maltose are not fermented. There is no change with milk. Potato gives a pasty black growth with folds. Glucose and maltose show an abundant, brownish-black, pasty growth with lighter colored filamentous edges. Injections into the peritoneal cavity of guinea-pigs were negative.

V. OTHER FORMS OF BLASTOMYCOSIS.

This disease of the skin is especially interesting in view of the presence in these Islands of a blastomycotic disease of horses and of the finding of blastomycetes in ulcers, in sprue and allied diseases.

Strong, in 1904, identified as blastomycotic a disease of horses known as pseudo farcy or ulcerative lymphangitis. He obtained only a slight growth at that time. A similar disease has been known for some time in Italy, France, Algiers, Russia, and Japan. It was more prevalent in Manila in 1903 and 1904 than at present, but we have seen several good cases. Fig. 14 shows a horse which we observed at the ice plant through the courtesy of Veterinarian Bishop. Veterinarian McKinnen, quartermaster's department, kindly sent us some sterile pus from a native pony and this after a month on glucose-agar, gave a slight growth. On transplantation this grew more readily on the same medium, but slowly on maltose-agar and potato. Figure 15 shows the convoluted, brown growth on maltose-agar. At room temperature the slow, new growth at the edges is pure white. Microscopically (fig. 17) it shows branching, segmented hyphæ with rounded ends and a great many free, round forms. The organisms in the ulcerating lymph-glands are oval, and are more uniform in shape and size than those in the skin disease; the membrane is not as distinct. The cultural characters are also very different. However, in order to determine if possible the question of relationship, two horses were inoculated subcutaneously, one with scales of the second form of disease, and one with the culture obtained from the third form; no infection resulted.

We have seen no general infection with blastomycetes, but think it probable that such cases exist in the Islands. We have had a Scout sergeant under observation with a good history and who was otherwise healthy, who suffered from gradual loss of weight, with cough, spitting of blood and pain in the chest. No tubercle bacilli could be demonstrated by repeated examinations or by inoculation; no ova were present in the sputum. Blastomycetes, however, were found in large numbers. The patient recovered completely in two months on ascending doses of potassium iodide. We have seen several cases like this one, but have not been able to satisfy ourselves entirely that the blastomycetes were pathogenic or to obtain encouraging cultures. However, we believe that lung infection may occur and may explain many obscure cases.

Dantec has recently stated that sprue is a blastomycosis of the intestinal tract and we have found numerous blastomycetes in two cases of sprue seen since reading Dantec's article. In two cases of hill diarrhœa we have encountered large numbers of blastomycetes in the stools and using Dantec's technic have obtained the same culture from each case. The growth is white, powdery, with a pasty center consisting of yeast-like forms and filamentous borders. This subject will be reported upon at length later. The ulcerations as reported by Strong, Sakurane and Okugawa in which blastomycetes have been found were undoubtedly due to these organisms. Those described by Strong resemble more closely the organisms from the horse than those which we have described. No

definite description of the organisms is given by Sakurane and Okugawa. The patient was a Japanese girl age 9 who had three ulcerating nodules on the face and nose and an enlarged submaxillary gland. Cultures from all the lesions were black, with gray filamentous edges and consisted mostly of yeast-like bodies which grew best on sugars.

VI. CONCLUSION.

In the Philippines a blastomycotic infection of the skin is one of the common dermatologic findings. It exists in at least three forms, two of which are milder than those seen in the United States. It is usually unrecognized; in the milder forms it is taken to be ring-worm or some form of dhobie itch; in severer types it is diagnosed as tuberculosis or syphilis. The milder types yield to local antiseptics, the more chronic and severe only to potassium iodide given internally. Further development of the subject will be necessary before these types, as well as those found in horses, in ulcers, in sprue and hill diarrhœa can be definitely classified.

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ILLUSTRATIONS.

PLATE I.

FIG. 1. Case I.
2. Lesions of four months' duration on nose and border of jaw.
3. Case V.
4. Case VI.

PLATE II.

FIGS. 5 and 6. Blastomycetes in scales, Case I, × 560.
FIG. 7. Section of skin, Case I, × 40.
8. Section of skin, Case V, × 50.

PLATE III.

FIG. 9. Section of skin, Case V, × 400.
10. Potato culture, Case IV; maltose-agar, growth from horse.
11. Growth on potato, × 350; Case IV.
12. Growth on maltose-agar, × 270; Case IV.

PLATE IV.

FIG. 13. Growth on maltose-agar Case IV.
14. Growth on agar; Case V.
15. Horse with ulcerative lymphangitis of neck.
16. Growth on glucose-agar, from horse; × 300.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

PLATE I.

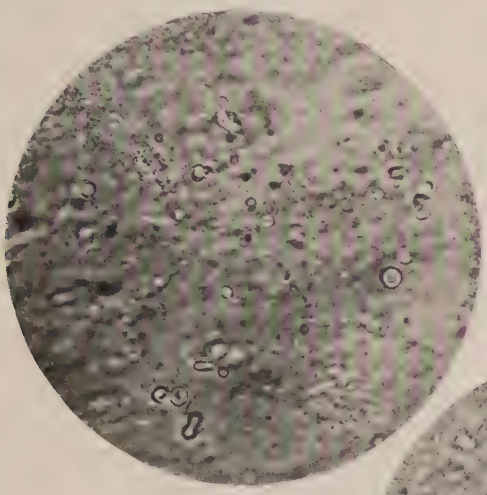


FIG. 5.

FIG. 6.

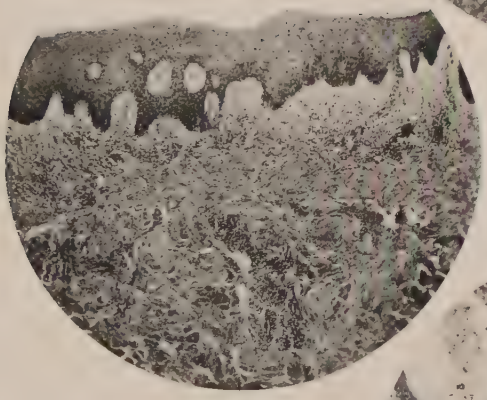


FIG. 7.

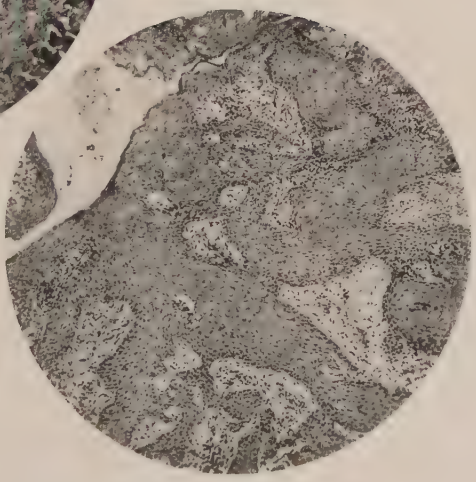


PLATE II. FIG. 8.

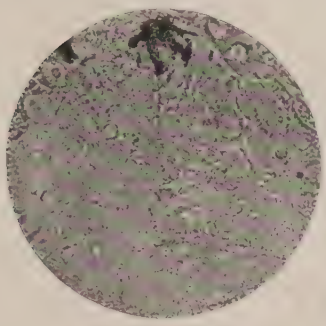


FIG. 9.

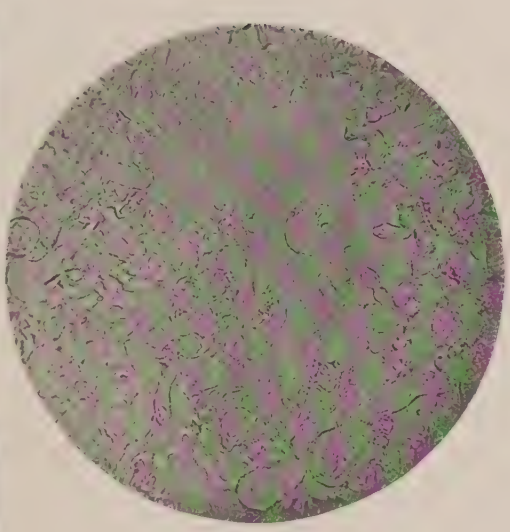


FIG. 11.

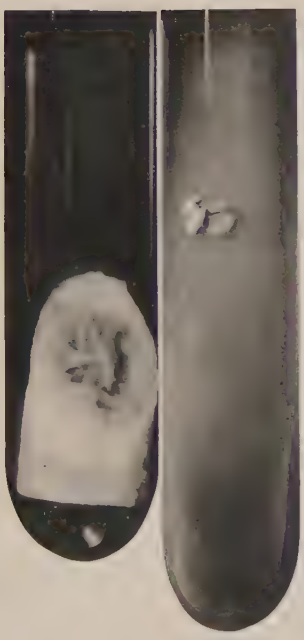


FIG. 10.

FIG. 12.

PLATE III.

FIG. 13.

FIG. 15.

FIG. 14.

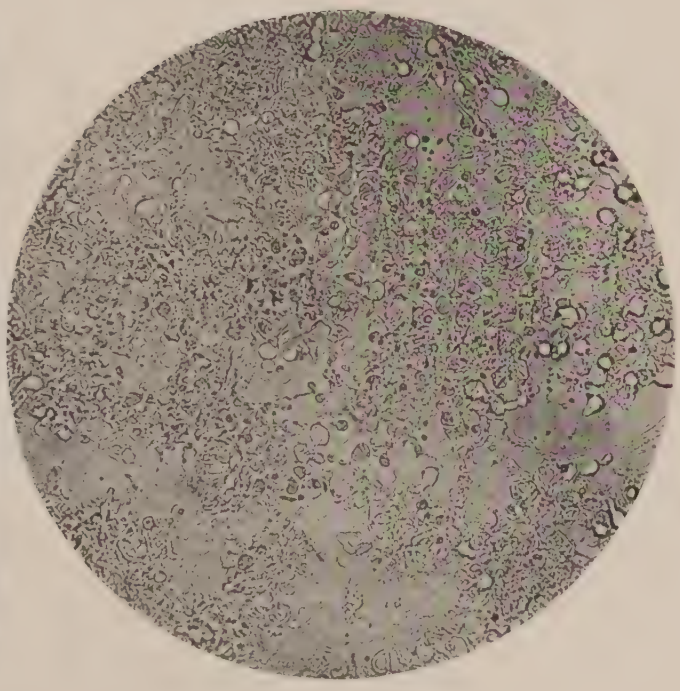


FIG. 16.

PLATE IV.

A REDUCTION IN THE COST OF ANTICATTLE-PLAGUE SERUM.

By E. H. Ruediger.
(*From the Serum Section, Biological Laboratory, Bureau of Science, Manila, P. I.*)

The cost of virulent material used in the preparation of serum against cattle plague is a question of great importance and one which has attracted my attention during the past few years. Numerous attempts to cultivate the specific organism of this disease have been futile, and it has been found necessary to adhere to the old method of bleeding to death bullocks suffering from cattle plague and to inject their blood, commonly known as virulent blood or V. B., into the animals to be immunized for serum purpose.

The average cost per bullock used for virulent blood is 50 pesos, Philippine currency (25 dollars, United States currency), and each bullock yields about five liters of blood, the carcass being waste; therefore, each liter of virulent blood costs 10 pesos.

In the course of the experiments carried on with filtrates of cattle plague material, I was deeply impressed (on repeating the experiments of Nicolle and Adil-Bey) by the high virulence of the artificial peritoneal fluid. The virulence of the peritoneal fluid seemed to be greater than that of the blood.

Having thoroughly convinced myself that the artificial peritoneal fluid is highly virulent, I requested Doctor Shealy of the Bureau of Agriculture, Manila, P. I., to inject 5 liters of a 0.5 per cent solution of potassium citrate into the peritoneal cavity of the sick bullocks, to bleed them to death an hour later, and to collect the peritoneal fluid and use it in immunizing serum animals.

Two series of animals, X and Y, were immunized for serum. The bullocks in series X received subcutaneous injections of artificial peritoneal fluid and the bullocks in series Y received subcutaneous injections of virulent blood. After having been well immunized the bullocks were bled for serum. The serum derived from the bullocks in series X was designated as serum X and that derived from the bullocks in series Y was designated as serum Y.

Ten bullocks, nonimmune to cattle plague, were now divided into two series, X and Y, with five bullocks in each series. The potency of serum X was determined on the bullocks in series X, and the potency of serum Y on the bullocks in series Y as follows:

SERIES X.

Five bullocks, numbered 78, 79, 80, 81 and 82, received 0.5 cubic centimeter of virulent blood injected under the skin and varying quantities of serum X.

Bullock No. 78, used as control, received no serum and died of cattle plague on the tenth day after inoculation. (Chart No. 78.)

Bullock No. 79 received 15 cubic centimeters of serum X. It went through an attack of cattle plague of moderate severity and recovered. (Chart No. 79.)

Bullock No. 80 was given a subcutaneous injection of 30 cubic centimeters of serum X. On the fifth day after inoculation reaction manifested itself by a rise of temperature. On the eleventh day the temperature dropped to normal and the animal made an uneventful recovery. (Chart No. 80.)

Forty-five cubic centimeters of serum X was injected into bullock No. 81. Five days later the temperature rose to 40°.5 C., but soon dropped to normal and the animal recovered without having shown any clinical signs of cattle plague. (Chart No. 81.)

Bullock No. 82, having received a subcutaneous injection of 60 cubic centimeters of serum X, showed a more marked reaction than did bullock No. 81 with the smaller dose of serum. Clinically he had loss of appetite for a few days. Diarrhœa never set in and recovery was rapid. (Chart No. 82.)

SERIES Y.

Bullocks numbered 83, 84, 85, 86 and 97, were each inoculated with 0.5 cubic centimeter of virulent blood and received varying quantities of serum Y.

Control bullock No. 83, which did not receive any serum died on the eighth day after inoculation. (Chart No. 83.)

Bullock No. 84, having received 15 cubic centimeters of serum Y, developed typical cattle plague and died on the tenth day after inoculation. (Chart No. 84.)

Thirty cubic centimeters of serum Y were injected under the skin of bullock No. 85; this animal died of cattle plague on the fourteenth day after inoculation. (Chart No. 85.)

Bullock No. 86 received an injection of 45 cubic centimeters of serum Y. After passing through a reaction of moderate severity, this animal made an uneventful recovery. (Chart No. 86.)

Bullock No. 87, having received 60 cubic centimeters of serum Y, had rather a severe reaction. Diarrhœa set in on the eleventh day after inoculation and continued throughout the twelfth day. On the twelfth day the temperature dropped to normal and remained normal; the diarrhœa suddenly subsided and rapid recovery followed. (Chart No. 87.)

CONCLUSIONS.

1. On comparing the results obtained with serum X with those obtained with serum Y, it was found that serum X had by far the higher potency.

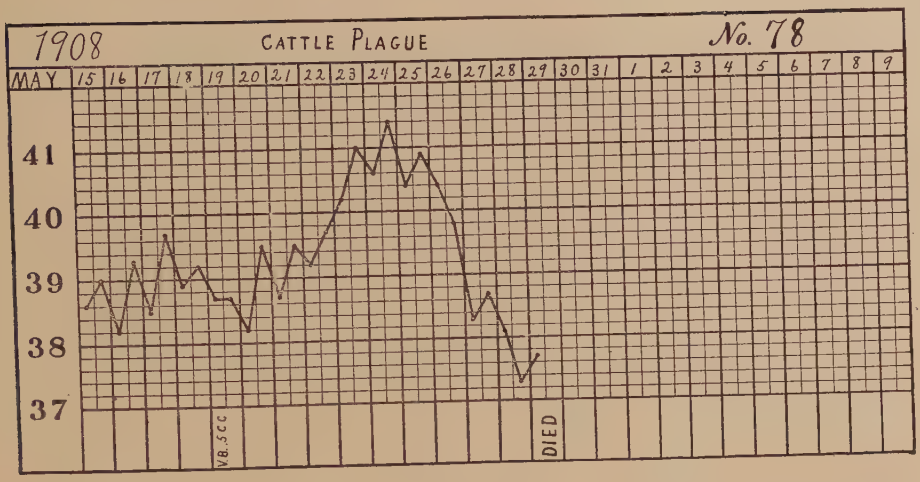
2. The quantity of virulent material used for inoculating serum

animals can readily be doubled by injecting one-half of one per cent solution of potassium citrate into the peritoneal cavity of the virulent blood animal and collecting this artificial peritoneal fluid after the animal has been bled to death.

3. The bullocks here used for virulent blood weigh between 200 and 250 kilos, each costs 50 pesos and yields about 5 liters of blood, each liter of blood costing 10 pesos. Since using the artificial peritoneal fluid, we obtain 5 liters of blood and 5 liters of peritoneal fluid, in all 10 liters from each bullock, at a cost of 5 pesos per liter.

ILLUSTRATIONS.

Chart Nos. 78 to 87.


1908
CATTLE PLAGUE
No. 78
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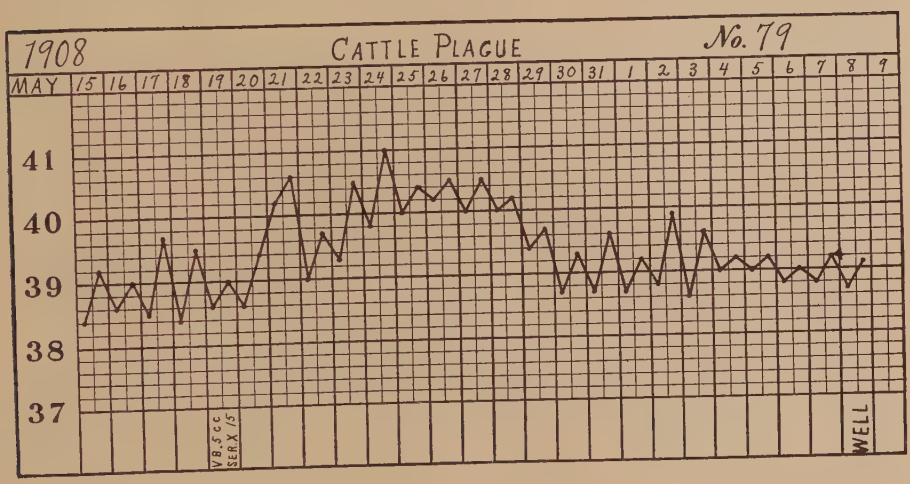
41
40
39
38
37
VB. 5 cc
DIED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No. 79
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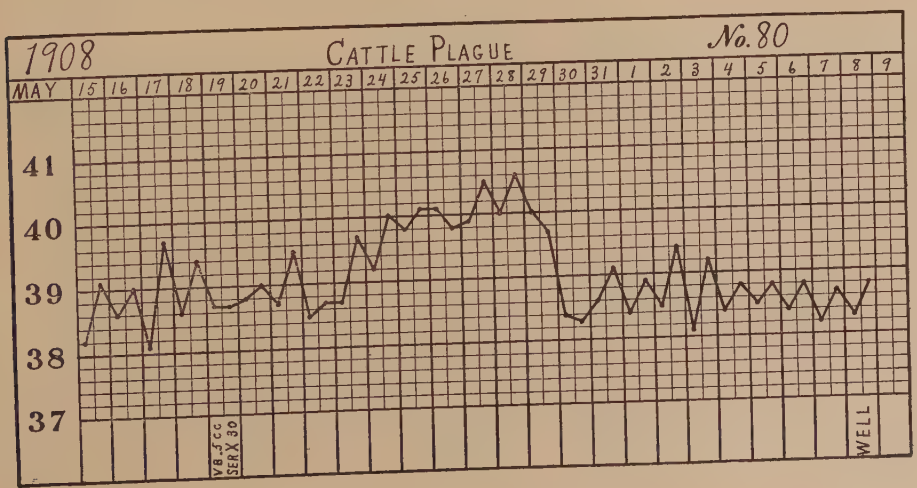
41
40
39
38
37
VB. 5 cc SER. X 15
WELL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CATTLE PLAGUE
No. 80
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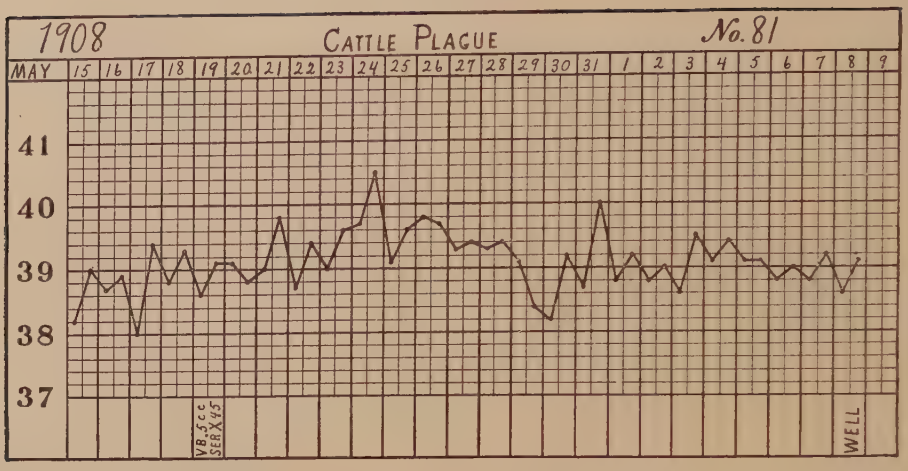
41
40
39
38
37
VB. 5 cc SER. X 30
WELL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CATTLE PLAGUE
No. 81
MAY
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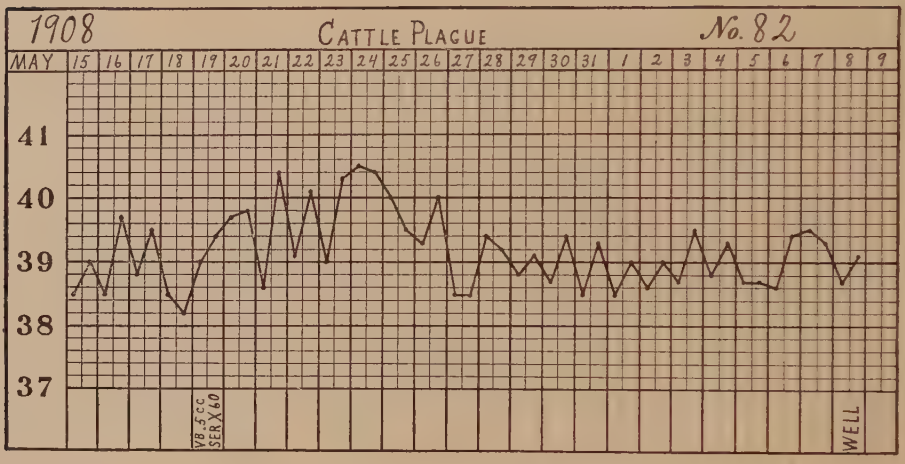
41
40
39
38
37
VB.5 cc SER X 45
WELL


1908
CATTLE PLAGUE
No. 82
MAY
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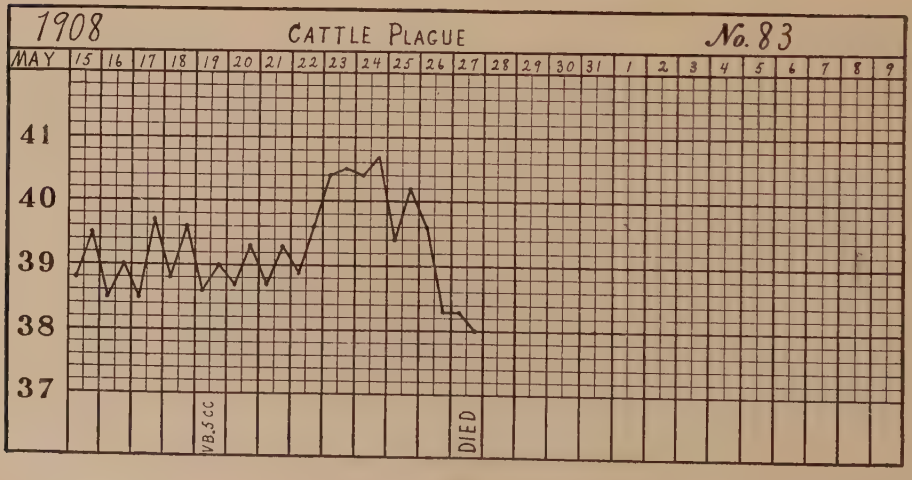
41
40
39
38
37
VB.5 cc SER X 60
WELL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MAY
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41
40
39
38
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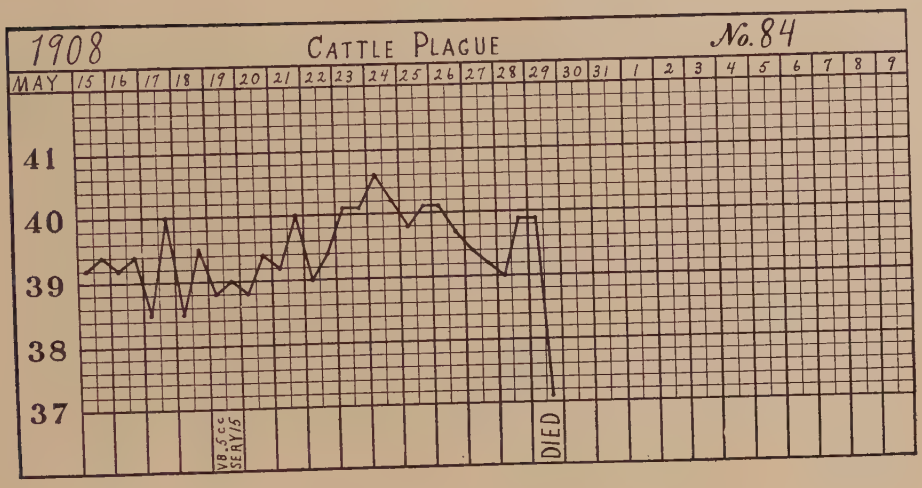
VB.5 cc
DIED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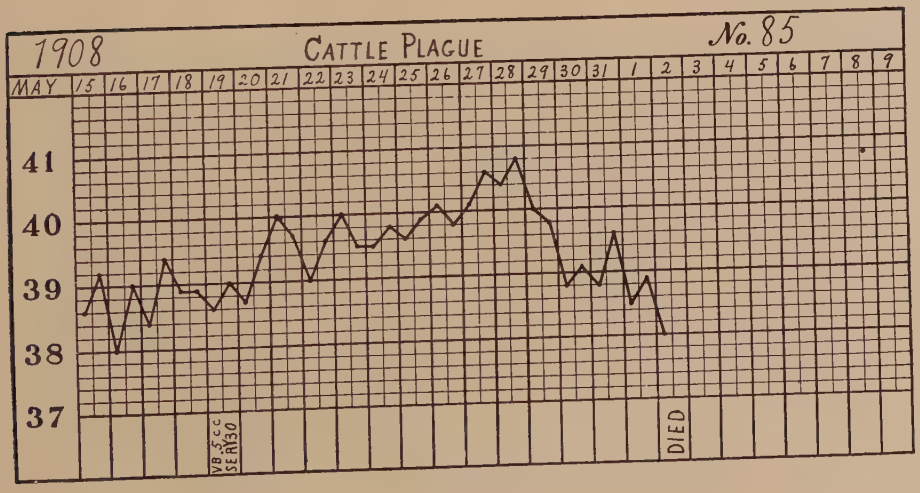
37
VB. 5 CC SER Y 15
DIED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CATTLE PLAGUE
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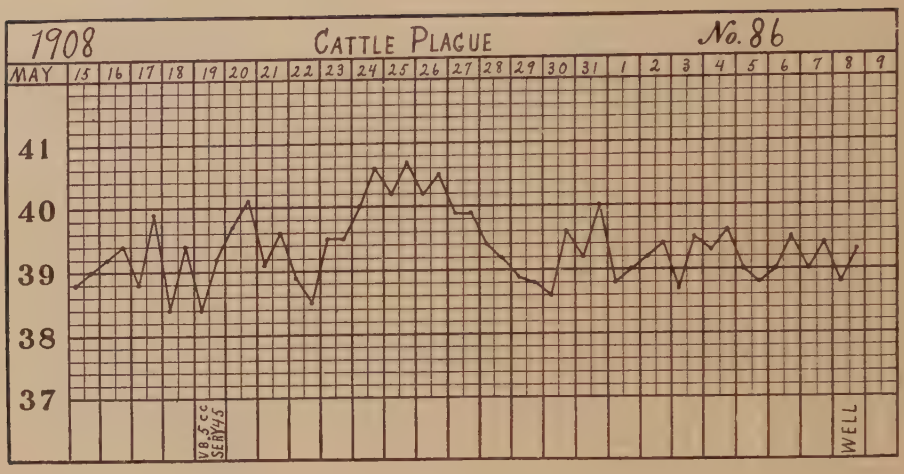
40
39
38
37
VB. 5 CC SER A 130
DIED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CATTLE PLAGUE
No. 86
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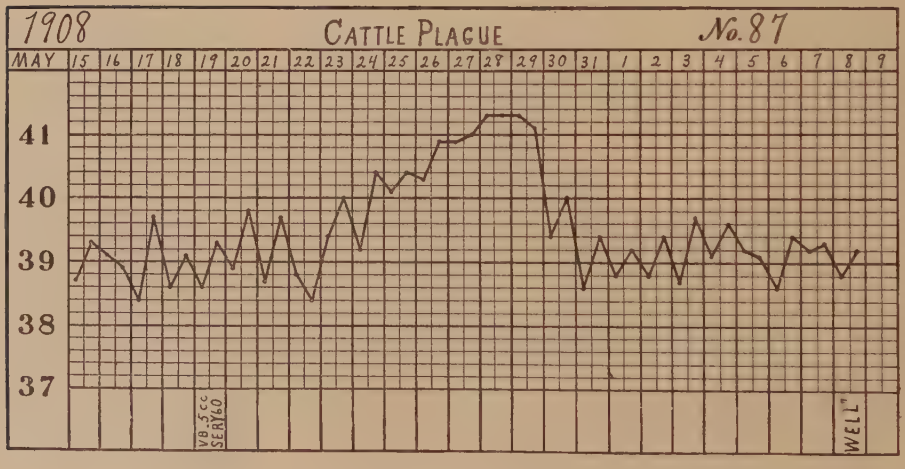
40
39
38
37
V.B. 5 cc SER 45
WELL


1908
CATTLE PLAGUE
No. 87
MAY 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9
41
40
39
38
37
V.B. 5 cc SER 60
WELL

RESULTS OF THREE HUNDRED EXAMINATIONS OF FÆCES WITH REFERENCE TO THE PRESENCE OF AMŒBÆ.[1]

By R. E. Hoyt.[2]

Microscopic examination of fæces was made a part of the regular laboratory routine at the Cañacao Naval Hospital in October, 1907, for the purpose of obtaining statistics as to the frequency of amœbæ and other intestinal parasites in the stools of officers and enlisted men of the Navy. Three hundred of these examinations were recorded in the latter part of November and the percentages given below are based on the findings in these cases. Records were also kept of blood examinations made from the first 200 patients whose fæces were examined, for the purpose of detecting or confirming any relation between blood findings and intestinal parasites. An occult blood test was made from the last three hundred cases with a portion of the fæces.

In order to make these stool examinations as nearly under the same conditions as possible, the following routine was carried out:

On the morning after admission a saline cathartic was given to those patients whose condition did not contraindicate its use. The first liquid stool was then sent to the laboratory for examination. Blood films from the patients whose fæces had been examined were then prepared, a differential white count made, the presence of parasites recorded, and in cases which indicated other blood disturbances, further tests were made, such as estimation of hæmoglobin, counts of red cells, etc. The chloroform, tincture of guaiac and turpentine test for occult blood was made in the last hundred cases examined.

It has been the opinion at this hospital that a distinction between the so-called *Entameba hystolotica* and *Amœba coli,* based on the points of difference first enumerated by Schaudinn, is practically impossible where specimens of crude fæces only are examined. While organisms showing these distinguishing characteristics may perhaps be recognized where cultures are used and a comparative study made of them, the fact remains that it is extremely difficult to pick out these supposed points of difference with any degree of accuracy on film preparations of fæcal matter. Therefore, no attempt at differentiation has been made. Furthermore, as the fæcal mass often contains cells which, under the microscope,

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, Manila, P. I., February 27, 1908.

[2] Passed assistant surgeon, United States Navy.

rather closely resemble non-motile amœbæ, specimens having positive amœboid movement at the time of examination have been recorded separately from those showing non-motile or encysted forms only.

Only 20 of the 300 patients whose stools were examined were admitted with the diagnosis of dysentery. Ten of these cases were either hospital corps men or medical officers stationed at the hospital, and in these cases the diagnosis was based more on the discovery of motile amœbæ in the stools than on any typical symptoms of dysentery.

About 45 per cent of the remaining cases might be classed as "medical," and these included fevers, chiefly malarial, dengue, and typhoid; mental and nervous diseases, diseases of the blood, intoxications and medical affections of the chest and abdomen. Diseases of the eye, ear, nose and throat were also placed in this group. Twenty-five per cent of the cases were "surgical" and included wounds, contusions, fractures, abscesses, tumors and the various surgical affections of chest and abdomen. Twenty-five per cent of the patients were afflicted with venereal diseases. Among all specimens of fæces examined, 104, or 34.6 per cent, contained motile amœbæ. Fourteen, or 70 per cent, of the specimens from the 20 patients admitted with dysentery were positive for amœbæ. Eliminating these 20 cases there remained 84, or 30 per cent, which gave positive evidence of motile amœbæ in the fæces, without showing at the time of examination any symptoms which could positively be attributed to their presence in the intestinal canal.

The following percentages were obtained as the result of these 300 examinations:

Motile amœbæ	34.6
Motile flagellates	15.3
Ova of *Ascaris lumbricoides*	10.0
Ova of *Trichocephalus dispar*	6.6
Ova of *Agchylostoma duodenalis*	3.3

Included in these 300 examinations were seventeen specimens from native Filipinos who were admitted to the hospital for various ailments, but only two were admitted for dysentery. The percentages of these cases are as follows:

Motile amœbæ	76.0
Ova of *Ascaris lumbricoides*	53.0
Ova of *Trichocephalus dispar*	76.3
Ova of *Agchylostoma duodenalis*	29.4

Subtracting these 17 cases from 300 and arranging the percentage for white enlisted men and officers of the Navy, the result is as follows:

Motile amœbæ	32.15
Ova of *Ascaris lumbricoides*	7.4
Ova of *Trichocephalus dispar*	2.5
Ova of *Agchylostoma duodenalis*	1.5

Sixty-six and two-thirds per cent of the dysenteric cases among white men showed positive evidence of motile amœbæ.

In addition to the records of motile amœbæ, an account was also kept of the presence of encysted and non-motile amœbæ, and in many cases where there was doubt of the identity of the organism the point was settled by examination with cultures. In 21 per cent of all cases examined either non-motile or encysted or both forms of the organism were found in preparations in which before no motile amœbæ could be detected. Allowing 5 per cent for possible error, there remains about 50 per cent of all cases examined showing evidence of amœbic infection.

An effort was made to discover from the first 100 cases some relation between length of service on the station and the presence of amœbæ in the intestinal canal, also any relation between sea and shore duty and infection.

From a total of 56 patients admitted from shore stations, station ships, torpedo boats and the smaller cruising vessels of the Philippine Squadron, from which the crew receive abundant liberty and were therefore often exposed to infection, 36 cases or 64 per cent showed positive evidence of amœbæ. Of 16 cases admitted from the larger cruising ships on which the men had spent one year on this or the China Station, 5 or 31 per cent were infected; of 21 patients admitted from the Armored Cruiser Squadron, which had been but four months on the station, 7 or 33 per cent showed amœbæ on examination. Sixty-five per cent of the patients admitted from strictly shore stations and 43 per cent of those admitted from strictly cruising ships gave positive tests for the organisms. Prolonged service on the station and continued residence on shore, therefore, as would naturally be expected, favors the chances of infection.

The examination of blood films, beyond showing a slight increase of eosinophiles in about 40 per cent of the cases containing amœbæ (over 5 per cent in 41 per cent of these cases) showed nothing remarkable. In nineteen preparations, malarial parasites were discovered, 13 from the blood of patients admitted with malaria and 6 from patients admitted with some other diagnosis.

The results of the tests for occult blood in the last 100 cases, while not conclusive in any way, are nevertheless interesting. Of the total of 35 cases found positive for motile amœbæ, 71.4 per cent gave a positive occult blood test. Seven of these cases were admitted with the diagnosis of dysentery; the others with diseases in which blood in the fæces would not be expected. In only 6 per cent of the remaining 65 cases was the test positive, the reaction being obtained in two *Agchylostoma* and one flagellate infection, and in one case of gall stones with cholocystitis.

During the eight months these examinations have been conducted, seven hospital corps men stationed at the hospital and three medical officers have been infected with amœbæ, although rigid precautions against such an accident were taken. In spite of the use of distilled water, cooked vegetables and carefully disinfected fruits, infection has occurred,

and it can therefore easily be understood how simple a matter it is for men who are stationed on shore in this vicinity and who take absolutely no precaution against the disease, to become infected in a comparative short time.

As before mentioned, while these officers and hospital corps patients were admitted to the sick list with the diagnosis of dysentery, the typical signs and symptoms of dysentery in nearly all cases were absent and the diagnosis was based chiefly on the results of stool examinations and lack of other evident cause for the symptoms present. As a rule, these symptoms began with gradually increasing debility, loss in weight and strength, anæmia, and in some cases digestive disturbances of various sorts; indigestion, slight diarrhœa or constipation and uneasy feelings over the region of the colon. In a few cases a mild neurasthenic state developed. Blood and mucus were not present in the stools and complaints of tenesmus were not made in these cases. Antidysenteric treatment (chiefly high irrigations of the colon), while causing an apparent relief of symptoms in some cases for a short time, resulted in no permanent good and eventually the patients whose terms of duty did not expire within the eight months, were surveyed and sent to the United States.

Some few of the other patients admitted to the hospital with diagnosis other than dysentery gave histories somewhat similar to those described above, and such symptoms as debility, anæmia, loss in weight and strength and digestive disturbances could not be accounted for by the diagnosis on admission. In these cases the physical and laboratory examinations would fail to give any more positive results than the presence of motile amœbæ or flagellates in the stools. Many of these cases also seemed to be temporarily benefited by high irrigations of the colon.

It is uncertain as to whether the presence of amœbæ plays any part in the causation of these symptoms or whether they are unfavorably influenced by a tropical climate, as is commonly believed; but reasoning from a knowledge of the power of other forms of intestinal parasites to produce symptoms of a general nature, such as those already mentioned, and taking into consideration the reported findings at autopsy in many cases of amœbic infection without apparent symptoms of dysentery, it would be very unwise to ignore this organism as an etiologic factor in affections other than typical dysentery.

THE INOCULATION OF BACTERIAL VACCINES AS A PRACTICAL METHOD FOR THE TREATMENT OF BACTERIAL DISEASES, WITH SPECIAL REFERENCE TO THE TREATMENT OF INFECTIONS DUE TO THE GONOCOCCUS.[1]

By Eugene R. Whitmore.[2]

None of us would think of treating a case of diphtheria without antitoxin, any more than we would think of opening an abdomen without taking precautions as to asepsis. It will undoubtedly be a surprise to many when I say that we are all neglecting every day a well founded method of treating a much larger class of bacterial diseases than the one that yields to antitoxins; and all earnest workers who are using this method, opsonotherapy, are obtaining as surprisingly good results as we get from antitoxin in diphtheria.

The great amount of literature that has been produced on Ehrlich's theory, the voluminous discussions on aggressins, agglutinins, precipitins, etc., the great amount of theoretical work on serum therapy, and the publication in the United States of a number of technical and abstruse papers on opsonotherapy, have made the every day physician lose all interest in discussions on biologic medicine. It is quite probable that too much stress has been placed on the taking of the opsonic index and it is possible that the theory may have to be modified, but the method of treatment based on this theory is giving results that are little short of wonderful.

Denys observed, in 1895, that in rabbits immunized against a virulent streptococcus, the leucocytes possessed markedly increased phagocytic power toward those cocci, as compared with the leucocytes of a normal rabbit. Several investigators confirmed this finding and Mennes showed that the same thing is true for the pneumococcus. Since that time considerable work has been done along this line, and several methods of bacteriologic diagnosis, notably the Widal reaction, have been developed on the basis of the detection of the products of immunity developed in the blood as the result of auto-inoculation.

Many investigators, notably Metchnikoff, claimed that the increased phagocytic power of the leucocytes when mixed with the immune sera was due to a

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, Manila, P. I., February 27, 1908.

[2] Captain and assistant surgeon, United States Army; pathologist, Division Hospital, Manila.

direct stimulation of the leucocytes. Wright and his assistants showed that the same phenomenon was not a result of the stimulation of the leucocytes, but is caused by an increase of some element in the blood which acts directly on the bacteria and so alters them that the leucocytes are able to ingest them. To these substances (for he showed that they were multiple) he gave the name of "opsonins." Thus, as Ohlmacher puts it, "it remained for Wright so to modify the vaccine of Pasteur as to arouse in the serum of Buchner a substance which prepared the disease producing microbe for ingestion by the phagocyte of Metchnikoff; thus bringing to practical humanitarian usefulness the laboriously studied theories of these pioneers in biologic therapy."

While Wright's work and his results have been well known for about four years, his method of treatment has been considered too technical for the average worker, and on this account it has not received the general consideration that it merits. It is not my intention to enter into a discussion of the principles of opsonotherapy, but I desire to urge that it is a practical method for the treatment of certain bacterial diseases, and the practice is the truly valuable condition we have in opsonotherapy.

Wright enumerates "the methods, other than vaccine therapy, which we have today for the treatment of bacterial diseases, as: (1) chemical antiseptics; (2) extirpation of the focus of infection; (3) the determination of lymph to the focus of infection; (4) sero-therapy; and (5) expectant treatment."

The mere enumeration of these methods calls to the mind of every one of us numerous failures, and how in any case these methods leave the issue more or less to chance.

Before proceeding to a discussion of the practical application of vaccine therapy it will be well for us to review the methods used by the body in the prevention and cure of bacterial disease.

(*a*) Mechanical covering. This is of comparatively slight importance.

(*b*) The main constituents are the antibacterial elements in the blood. These are (1) bacterial substances, which kill bacteria; (2) bacteriolytic substances, which kill and dissolve bacteria; (3) agglutinins, which agglutinate bacteria; and (4) opsonins, which alter the bacteria so that they are readily ingested and digested by leucocytes. In this connection the leucocytes must be considered as of great importance.

(*c*) The body is able to manufacture antitoxins which do not necessarily affect the invading bacteria, but neutralize the toxins produced by these bacteria.

The body combats bacterial invasion in two ways: The first and most important, by attacking the bacteria themselves and the second by neutralizing the toxin produced by the bacteria. The diphtheria bacillus is the shining light among the organisms which stake their existence against an antitoxic combat; while the tubercle bacillus, the pyogenic cocci, including the gonococcus, the pneumococcus, the typhoid bacillus and a number of others stake their existence against an opsonic combat.

Failure to recognize these different methods by which the body combats bacterial invasion has been the cause of many bitter disappointments in the field of serum therapy.

So far it has been impossible to demonstrate any increase of opsonin for the diphtheria bacillus by inoculation with the dead bacteria. Practically, however, it would seem that some antibacterial element must be developed, or increased, for it is hard to understand why the local condition ceases so promptly and why the diphtheria bacillus regularly, though slowly, disappears from the throat if the reaction is purely antitoxic. On the other hand, it seems probable that some antitoxin is produced against the bacteria that are mainly attacked by opsonins. This would account for some of the partial successes achieved in the antitoxic treatment of bacterial diseases that are now known to belong in the opsonic army.

Again to quote Wright's words, "it is only when bacteria or their products enter the blood and endanger life that nature addresses herself seriously to the task of immunization. As long as a bacterial invasion is purely local, nature opposes to it no more than a passive resistance." This is an important generalization and we at once see that it is the key to the situation in lupus, acne, furunculosis, gonorrhœal arthritis, epididymitis, urethritis, conjunctivitis, and a number of other infections. Further, the production of antibacterial and antitoxic substances following inoculation is a local process at the site of the inoculation.

With these few remarks on the physiology of immunization, we can proceed to a discussion of the specific application of vaccines in the treatment of bacterial diseases. In order that we may successfully combat bacterial invasion by use of its opsonins which are normally present in the blood, two things are necessary: (1) the opsonins must attack the bacteria and so alter them that they can be ingested by the leucocytes; (2) the leucocytes must ingest and disintegrate the bacteria. This at once gives us the key to the opsonic index which, in the case of bacterial disease, is an index to what nature is doing toward immunization against the specific bacterium. "Taking the opsonic index" is a fairly technical procedure and has been the main stumbling block in the practical use of opsonotherapy. It was not my intention to describe the means of taking the opsonic index, but there seems to be so much misunderstanding about it that I will give the method used in practical work.

Ordinarily the bacteria are grown on agar. The gonococcus does not grow on this medium, so I have cultivated it by taking a drop of blood from a man's ear in the usual way, smearing the blood over the surface of a plain agar or a glycerine-agar tube and making my culture on it. I have made hundreds of cultures in this way during the past twenty months and have very rarely had a contamination. It is also very easy to obtain a pure culture of the gonococcus in this way from an early case of urethritis. I have been able to induce some strains of the gonococcus to grow on glycerine-agar after a long time.

Equal volumes of the patient's blood (from say a case of gonorrhœal arthritis),

drawn directly from the ear; a 1 per cent sodium citrate solution in normal salt; and a normal salt emulsion of a young culture of gonococcus, are mixed in a pipette and put in the incubator at 38° for twenty minutes. At the end of this time the mixture is blown out on slides, stained, and the number of cocci ingested by, say, 100 leucocytes are counted and averaged. Suppose the average is two. Exactly the same procedure is gone through using a normal man's blood instead of the patient's. Perhaps here the average is four. This normal man's opsonic index is taken as 1. So, since the average number of cocci taken up by the patient's leucocytes is one-half the average number taken up by the normal man's blood, it follows that the patient's opsonic index is 0.5.

We see that the opsonic index is purely a matter of relative amounts of opsonins in two samples of blood, one of which is normal. The temperature at which the reaction is carried on may vary from 37° to 39° and the time from fifteen to thirty minutes, but both bloods must be treated exactly alike as to temperature and time.

I will not enter into a discussion of Simon's method of determining the percentage of phagocytic leucocytes, or the relative merits of the two methods. One of my main objects, as I said above, is to impress the fact that the taking of the opsonic index has nothing to do with the actual treatment of the case; that in certain bacterial diseases, enough has been done to place opsonotherapy beyond the experimental stage; and that the dosage has been determined accurately enough so that the vaccine can be inoculated and the results of treatment determined by the progress of the case. Thus, while the determination of the opsonic index is the only scientifically accurate method of following the effect of the inoculation, practically, in some diseases the progress of the case is a sufficient guide as to the effects of the treatment. Again, since it has been shown that killed cultures of bacteria retain their opsonin producing properties for weeks, the actual manufacture of the vaccine is not a necessary part of the treatment of a particular case.

Wright used Koch's new tuberculin in the treatment of local tuberculosis. Burroughs, Wellcome and Company have placed on the market vaccines for use against typhoid and staphylococcus infections. Suppose we have a case of furunculosis to treat. Enough work has been done already on furunculosis so that we can be sure of obtaining a pure culture of *Staphylococcus aureus* from the furuncles in our case and of finding our patient's opsonic index low for that particular coccus; we can also be certain that the inoculation of half a billion to one billion dead staphylococci of this strain would cause a rise in our patient's opsonic index and at the same time the furunculosis would clear up. This being the case, it is necessary to obtain a tube of *Staphylococcus aureus* vaccine, inject the proper dose, as given on the tube, and determine the result by the progress of the case.

Thus we see that the taking of the opsonic index is one separate manipulation and the preparation of the vaccine is another, and the only absolutely essential step in the treatment of any case of certain of

the bacterial diseases is the inoculation of an appropriate dose of the proper strain of vaccine; just as the only absolutely essential step in the treatment of a case of diphtheria is the injection of an appropriate dose of antitoxin that has been prepared by some other person who has proper facilities for such preparation.

This is especially true in the treatment of gonorrhœal arthritis and epididymitis. Here the vaccine must be prepared from a gonococcus obtained elsewhere than from the patient, as it is impossible to secure a pure culture of this organism from him under these conditions. So the vaccine can be prepared by some one having proper facilities ten or ten thousand miles away. It is necessary to mix the three or four strains of gonococcus known in opsonic work, standardize the vaccine, and send it to the man who is to treat the case. He injects the vaccine and determines the result. If considered necessary from the clinical condition, the dose can be repeated at intervals of seven to ten days with the assurance that it can do nothing but good. Two to five doses are generally sufficient for the most intractable cases of gonorrhœal arthritis and often the result at the end of 24 hours after the first injection is little short of marvelous.

My experience with epididymitis and urethritis is of too short duration to warrant any statement at present, although I have seen cases of epididymitis clear up in twenty-four hours, and one para-urethral abscess which I observed disappeared almost entirely in 36 hours. Ohlmacher has reported good results in two cases of epididymitis.

The condition in urethritis is more complicated, as here we usually have a mixed infection, and it is known that inoculation against one organism in a mixed infection may actually give the other organism a chance to thrive. Some apparent success, however, in urethritis encourages me to continue my work.

Chart I is taken from a case of furunculosis which shows the negative and positive phases in the opsonic index as well as the subsequent decline of the curve toward normal. Clinically, all furuncles began to heal and no new ones appeared after twenty-four hours following the first inoculation.

Chart II is compiled from a case of gonorrhœal arthritis. It shows a prompt and marked increase in the opsonic index for the gonoccoccus, and clinically twenty-four hours after injection the man was free from pain and tenderness in a joint that had been extremely painful and tender for nearly four months. Some stiffness and enlargement remained as a result of the prolonged inflammation, but these conditions rapidly subsided and the man expressed himself as able to do his duty in less than one week after his first injection. He was kept in the hospital another week to observe his index and the practical result of the treatment. He was allowed to go on duty two weeks after his first injection, as he was entirely well.

Other cases of gonorrhœal arthritis, furunculosis, acne, empyema and lupus have given excellent results. I have just injected a refractory case of *Pemphigus contagiosa* with vaccine from the pustules of the patient and the outcome, apparently, will be satisfactory.

The result in gonorrhœal ophthalmia should be good, and the ability to keep vaccine on hand would make it possible to start opsonic treatment at once without in any way interfering with the other treatment of the case. Ohlmacher reports good results in three cases.

SUMMARY.

(1) The opsonins are the most important of the antibacterial elements in the blood and opsonotherapy is one of our best methods for the treatment of an ever increasing number of bacterial diseases.

(2) Opsonotherapy has passed the experimental stage and since it has been shown that certain vaccines retain their potency for weeks, it is practicable to treat certain bacterial diseases by the injection of the corresponding vaccine, just as we treat diphtheria by the injection of the corresponding antitoxin.

(3) Gonorrhœal arthritis responds readily to inoculation with vaccine, and this condition is to be placed in the list of diseases which can be treated by a vaccine prepared commercially.

(4) Gonorrhœal epididymitis seems to respond readily to inoculation with vaccine, while, so far, no statement can be made regarding urethritis.

(5) The opsonic index, while it is the only scientifically accurate method of estimating the immunization response to bacterial inoculation, is not necessary in the treatment of certain bacterial diseases; the progress of the case being sufficient index for practical purposes in such diseases.

(6) Bacterial inoculation does not interfere in any way with any other treatment that it is desired to carry out in a particular case of any disease.

ILLUSTRATIONS.

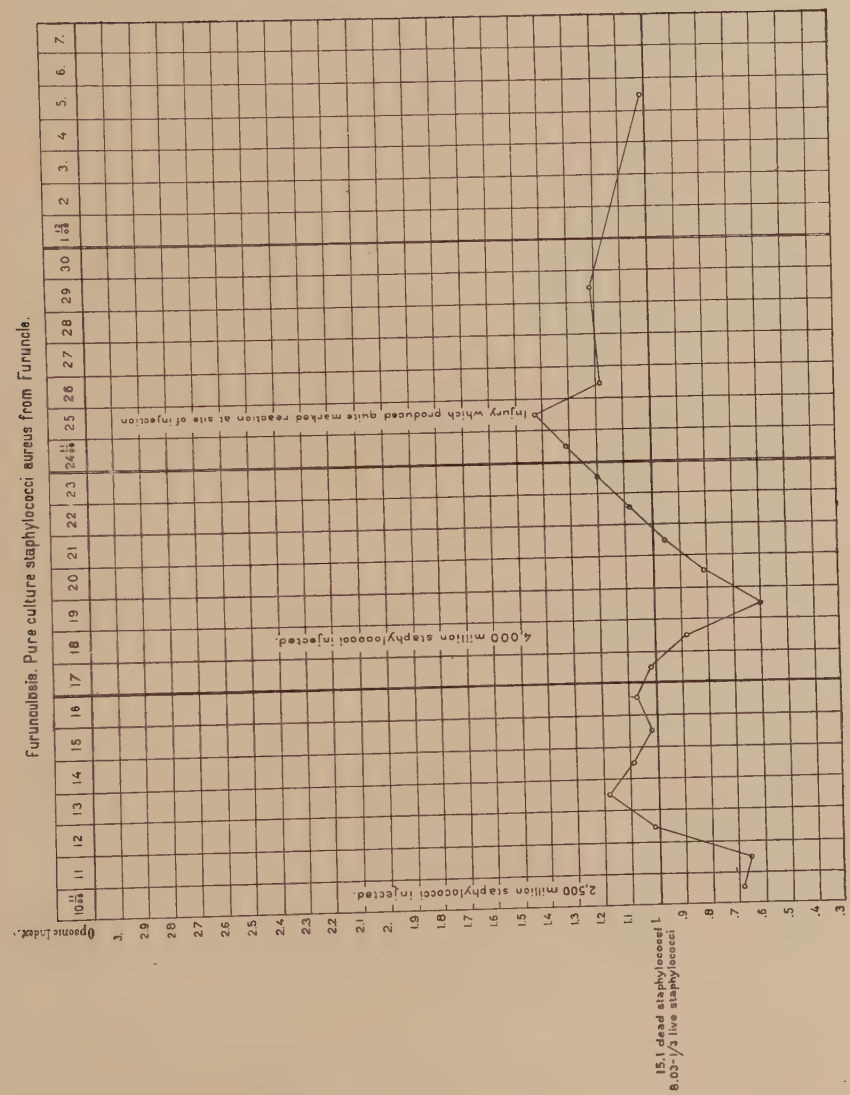
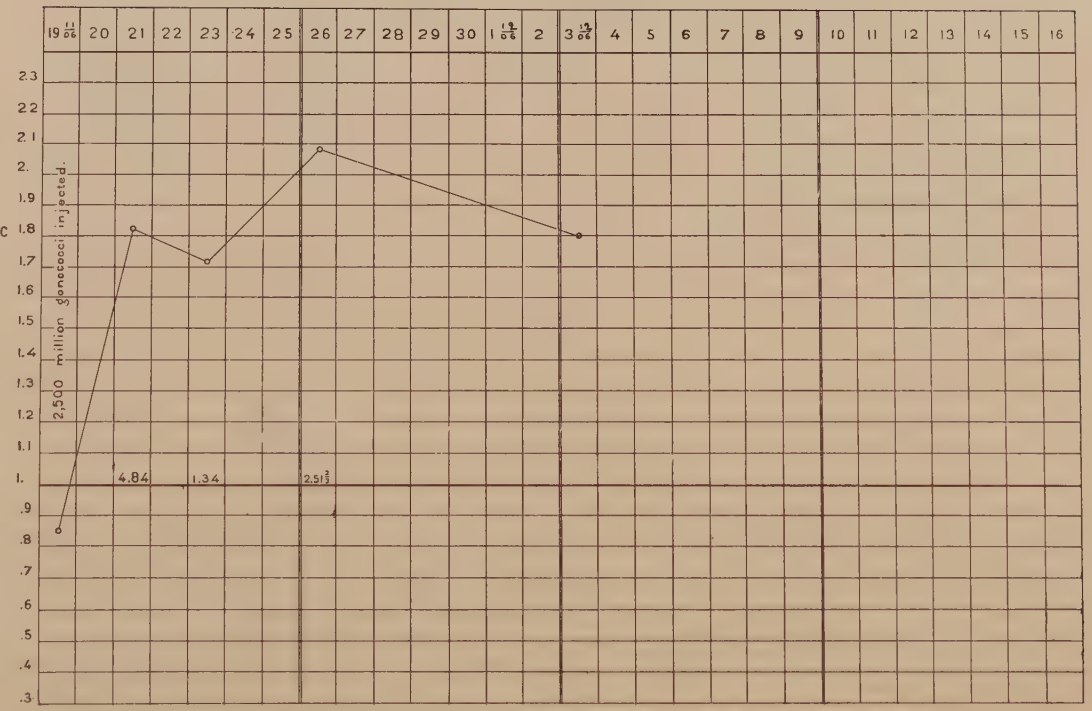

Furunculosis. Pure culture staphylococci aureus from Furuncle.
Opsonic Index.
2,500 million staphylococci injected.
4,000 million staphylococci injected.
Injury which produced quite marked reaction at site of injection
15.1 dead staphylococci
8.03+1/3 live staphylococci

CHART I.

CHART II.

Gonorrhoeal arthritis.



19–11–06 opsonic index .86+. 21–11–06 opsonic index 1.83+.
23–11–06 opsonic index 1.72+. 26–11–06 opsonic index 2.09–.
3–12–06 opsonic index 1.8.

SANITARY CONDITIONS AND NEEDS IN PROVINCIAL TOWNS.[1]

By Thomas W. Jackson.[2]

The circumstance of continued residence in provincial towns, (where the opportunities for observation of social and sanitary conditions surpass those for studies purely medical), is the principal reason for my choice of this topic. Some members of the Philippine Islands Medical Association have spent a good portion of the past ten years in these Islands, others have had interrupted residence, and some have returned after five years' absence or longer in America. To this last class, at least, a comparison of conditions then and now is interesting.

In Manila, at every hand, striking changes in methods and facilities for transportation, communication, procuring of food, recreation, illumination, and external conditions affecting the comfort and safety of living for Americans are in evidence. Great improvement in highways, buildings, sewers, hospitals, schools and churches, and bettered conditions as to the control of epidemic diseases are notable.

A splendid Bureau of Science and a Medical College have been established. Exhaustive studies of tropic diseases have been undertaken and important facts concerning their causation have been discovered. In a word, great things have been wrought.

Both in and out of Manila, American soldiers and sailors are so safeguarded that the morbidity rate for the Islands is slightly less than that for the United States. Conditions are less favorable for Americans outside of garrisons, but with due attention to hygienic law and the observance of well-known precautions against infection, reasonable health may be maintained by all. The relative infrequency with which Americans have suffered from epidemic plagues, such as cholera, emphasize this contention.

The Director of Health for the Islands stated in the annual report for 1906 that "each succeeding year of experience in health work shows that the white man's chances of contracting disease in the Philippine Islands are less than in the United States." He also shows statistically that for

[1] Read at the Fifth Annual Meeting of the Philippine Islands Medical Association, Manila, February 29, 1908.

[2] First lieutenant, Medical Reserve Corps, Fort William McKinley.

the year 1906 the death rate, including those who died after returning to America, was not higher than in the most salubrious communities in the United States. He further states that "in no country in the world has the Government done more for the protection of its employees." Granting the correctness of these statements we may dismiss the white man from our consideration and turn our attention to conditions among Filipinos in the provinces. Inasmuch as 95 per cent of the Filipino people live outside of the city of Manila we may fairly take conditions outside of that city as an index of our success or failure in hygienic education at the end of nine years of American occupation.

Let us consider these conditions briefly. It would be unfair to inquire whether the improved conditions just recited for Manila obtain likewise in provincial towns. It is fair, however, to inquire in what respect sanitary conditions in provincial towns are better than they were at the time that American civil government was instituted in these Islands. I have lived in native houses in provincial towns for the past year, closely surrounded by Filipino neighbors and by day and night I have observed their customs and habits. Under the circumstances, indeed, it would be impossible to avoid an intimate knowledge of their manner of life. In 1901 and 1902 I had a similar opportunity and I therefore feel that I am qualified to make comparisons. The habits, customs, and manners of the people are sufficiently well known to most of the members of the Association and I have reason to believe that the conditions I have observed are neither better nor worse than those in the average provincial town. Putting aside matters which are purely æsthetic and fully recognizing the great progress made by the government in its efforts to provide for the people the things which make for peace and contentment, I am of the opinion that our efforts at sanitary education, if they have not failed completely, have been far from successful.

Accepting the testimony of my eyes I am forced to believe that in provincial towns conditions are practically as they were seven years ago, as regards such personal habits as defecation, urination, expectoration, and eating with the fingers. In less individual matters, affecting the community rather than the family, such as the condition of the market places, the contamination of foods by insects and animals and the disposal of garbage and other wastes, the improvement is inappreciable. There is sufficient improvement for remark in the single matter of relative tidiness of streets and dooryards. Even in this particular, which is cosmetic rather than vital from a sanitary viewpoint, one can point to little more than the disappearance of carabao wallows. It is true that in every town of any size there are people of comparative wealth and relative intelligence and education. Some of them have been educated in schools of our establishing. These people exhibit cleanliness of person and clothing, eat at tables with spoons and forks and give evidence of a

desire for better things for their countrymen. This part of the population as compared with the whole, however, is inconsiderable and it must be admitted that even in the case of the wealthy and intelligent, sanitary matters which really count are quite neglected.

Let us take for example a certain provincial capital less than one hundred miles from Manila. The population is between 8,000 and 10,000 people. The provincial government offices, prison, and high school are located here and the governor and members of the provincial board reside in the town. The town is situated upon the bank of a river which serves as a sewer and water supply, as a universal bath-tub for people and animals, and as a wash tub for the clothing of 10,000 people. Passing through the streets we encounter chickens, ducks, goats, half-starved dogs and unnumbered pigs, many of them with decorations of human fæces on their heads and backs, foraging for garbage or the droppings from passing horses and carabaos. Markets and *tiendas* expose their wares of fruits, cooked rice, fresh meats, fish, vegetables and crude sugar to the clouds of dust in the streets and flies swarm in thousands over the sticky sugar and bloody meats. Dogs and cats walk over the exposed vegetables and natives, with fingers whose condition may be imagined rather than described, handle the food *ad libitum*. Screens against flies are everywhere absent. Defecation is performed in public at all times upon the streets and river banks. With one exception, the water-closets in all homes but those of the Americans are of the pig-flushing variety.

Stagnant pools of waste water are found beneath many houses, serving as pig-wallows or as mosquito-breeding places. The ubiquitous and iniquitous pig feeds upon human excrement and in the end serves as food for the people. Nudity, under the law, is a punishable offense, but boys and girls of the age of pubescence parade the streets, practically naked, before the eyes of American women and children. In this town there are 27 Americans variously connected with the Civil Government, Educational Department, Constabulary, and Army. There is annually expended for salaries of American teachers stationed here (most of them in the provincial high shool) more than 20,000 pesos, Philippine currency, while for practical sanitation, exclusive of extraordinary expenditures for the suppression of epidemic diseases, I am unable to learn of any outlay. A native physician, formerly employed as municipal physician at 30 pesos per month, is now filling the office of district health officer. He draws a salary of 200 pesos per month. He is the sole official representative of sanitation and he is absolutely inactive so far as local conditions are concerned. There is no municipal physician.

It is interesting to know that upon the municipal statute books of this particular town there are ordinances, with penalties for their violation, covering practically all of the abuses which I have named. These

ordinances are absolutely dead letters. They are doubtless unknown to a large part of the populace. They provide for garbage collection and disposal and for a sanitary cart for the house-to-house collection of excreta and other wastes. They prohibit the running at large of pigs and other domestic animals and prescribe penalties for the violation of these provisions. They provide for the screening of foods in market places and for inspection and policing. In short, they provide for the maintenance of decent conditions, which is probably as much as we can reasonably expect in this country.

This recital is not made to excite commiseration for the Americans who by force of circumstances are obliged to live in these towns and endure these conditions; neither is it in any sense a complaint against the constituted Health Department of these Islands. If I sought to excite sympathy for the Americans I would relate conditions as they exist in another large town in the same province, where matters are worse than I have described them. As for the Public Health Department we can not overlook the tremendous work it is doing and has accomplished, especially in the sanitation of Manila, the establishing of vital statistical records, charity hospitals, the vaccination of the people, the control of epidemics, the segregation and colonization of lepers, and best of all the enactment of laws.

It is rather on behalf of the Filipinos themselves that I have described conditions as they are. The question which propounds itself is this: "Are we doing less than our duty to the Filipino people in this matter?" I fear that we are. Whether we agree with the doctrine that the United States is exercising a temporary control of the Islands preparatory to entire withdrawal therefrom, or whether we consider the Islands as colonial possessions, it must be admitted that at present the United States Government is actually in possession and control. As candid medical men and good Americans we recognize, as our duty, the obligation to promote health conditions in these Islands to our utmost. This obligation is not contingent upon the wishes of the Filipinos. We hear a great deal these days about altruistic government. In what way can real altruism be better shown than by requiring the observance of hygienic law?

In the last annual report of the Bureau of Health for the Islands attention is called to "the comparative freedom from disease which residents of the Philippine Islands who are willing to follow the few simple rules recommended by the Bureau of Health enjoy". In this statement lies the explanation of the situation. Residents of the Islands are "permitted a choice" as to whether or not they will observe hygienic laws, the violation of which affects not only themselves, but their fellow-men. So long as this is our policy we can hope for little improvement. Surely we can not hold the Health Department accountable. The Bureau must

reflect the policy of the Government. It has been stated that "the mere suggestion of interfering with the home life of the people would cause storms of protest."

As earnest men, seeking as a common end the betterment of humanity in these Islands, we can afford to consider well it seems to me whether a storm of protest should stand in the way of decency and the fulfillment of a pledge to the civilized world to lift these people from all that is destructive and degrading.

What are the probabilities of opposition on the part of the Filipinos to the enforcement of sanitary laws such as have been referred to in this paper? Doubtless there would be both silent and public opposition, but I do not believe that it could possibly attain to dangerous proportions or cause lasting disaffection. Witness the acquiescence of the people following the gambling laws and the sedition and flag laws. Where has there been effective or successful opposition to the enforcement of cholera regulation or the segregation of lepers? The present may well be called a period of the Reign of Law, and it seems to me that the present is the opportune time for sanitary reform. Moreover, there is no doubt that the educated class of Filipinos would conform to the laws and support them with influence, when once apprised of their importance, the necessity for compliance, and the unavoidable penalties of noncompliance. That the liberties of the people would be affected by such reform is not to be seriously contended.

When the provincial boards of health were abolished in 1906 there were vested in the district health officer the following duties and powers:

> General supervision and control over health and sanitary works and over municipal boards of health in the district; power to institute proceedings to abate nuisances and to prosecute violations of the law; to recommend to the Director of Health sanitary regulations for prisons, schools, and institutions; to make internal quarantine regulations; to attend all persons entitled to free medical attendance, and to compile statistics. District health officers are authorized to require owners, agents, and occupants of buildings to maintain sanitary conditions and are themselves required to call upon *presidentes* to enforce municipal laws.

Consider the very great possibilities of sanitary accomplishment under this law, if all district health officers were competent, energetic, fearless men, unhampered by political complications. No argument is needed to show that under existing conditions in the Islands few Filipinos in the provincial towns are qualified for these positions. Political intrigues and the fear of offending powerful interests disqualify many of those who have the necessary executive or professional attainments. To look for relief in sanitary matters through the pressure of native public sentiment created by the advice of Filipino physicians, as has been suggested, is chimerical I fear. Likewise, the introduction of sanitary teachings into the home through the school children must be a slow and

tedious process, unlikely to produce results within a generation. I have heretofore contended that the teaching of hygienic principles is a necessary and preliminary foundation for any material development of the people of the Philippines, and I am more than ever convinced that the enforced observance of basic hygienic laws must prevail before progress worthy of the name will be made.

The teaching of hygiene and sanitation has a place in the scheme of public education for the Islands and doubtless much good is accomplished thereby. The matter is a delicate one for the average teacher and is not given the prominence in the course, I suspect, that it deserves. In November last, at the invitation of the principal in charge, I gave two lectures to the pupils of the provincial normal school in session in one of the provincial capitals. These students were advanced pupils and native teachers of the primary schools and numbered about two hundred. Judging from the number of questions propounded after the lectures were over there seemed to be shown an encouraging interest, but an extremely elementary knowledge.

Doubtless these conditions will be overcome in due time. In the mean time we should try the effect of the enforcement of sanitary law.

I have not considered it necessary to attempt to show by argument, or by the citation of statistics, the relationship between filth and disease. The fact that the public health is indirectly affected by the insanitary practices of the people is well understood by a body of men of the character of this Medical Association. It would be difficult to think of many serious tropical diseases which may not be disseminated by some one or more of the practices which prevail here. Neither have I considered it necessary to discuss the view, somewhat widely held, that the scavenger pig is a blessing in disguise. We should insist at all times that he has no place among the sanitary forces of the Philippine Islands. Not only is he offensive from an æsthetic point of view, but he is entirely inefficient as an animate crematory or germ converter. That pathogenic bacteria and protozoa are rendered benign by a trip through the intestinal canal of a pig is an assumption absolutely without warrant.

As cited by the Director of Health, the pollution of streams is without doubt one of the greatest factors of disease production in these Islands and it is gratifying to read in the last annual report of the Health Bureau of a comprehensive plan to remedy in good time this most serious condition, involving as it does financial and engineering problems of magnitude. For the present, greater attention should be devoted to the matters of soil and food pollution which, it is believed by many, will prove to be nearly as great sources of disease as water pollution. Compared with the task of furnishing a pure water supply these problems should be easily solved. Apparently we already have sufficient law. It is not to be expected that miracles will be wrought by its enforcement,

but conditions can certainly be bettered. The Director of Health has stated that "with very little cost the hygiene of domestic life could be very much improved." I am of the opinion that the same statement holds good for municipal hygiene and doubtless we all agree with him that "efforts along this line would be well rewarded by the greatly decreased mortality which would be sure to result."

It seems to me that meetings of the character of this Medical Association are proper places for the discussion of questions of hygiene and sanitation. We feast intellectually upon masterly papers which present the purely scientific side of our profession. New and attractive vistas of possibility are unrolled before us, as suggestive hypotheses in explanation of problems hitherto baffling are presented.

It is neither belittling nor damaging to our dignity to consider the simpler questions of sanitation in the home and in the community. In so doing one is often obliged to pronounce opinions and to offer friendly criticisms which are apt to be misinterpreted as malevolent.

In conclusion I wish to state that I have abiding confidence in the motives, plans, and ultimate success of the Philippine Government in the great sanitary battle in which it is engaged. I believe that the phase of the problem which I have attempted to present is an important detail, worthy of attention now, but I am not unmindful of other phases nor of the magnitude of the work in hand and results already achieved.

EDITORIAL.

DISCUSSION OF THE PAPER BY DR. THOMAS W. JACKSON.

Dr. Victor G. Heiser, Director of the Bureau of Health of the Insular Government and professor of hygiene in the Philippine Medical School: I feel grateful to Dr. Jackson for presenting this paper because it gives me an opportunity to reply to a great many criticisms as to the manner in which local hygienic measures are carried out. The point of view makes a good deal of difference. Some persons think that it would be easy to remedy these unfortunate conditions. Those of us who have had most experience in dealing with them know that this is not true. Hygienic methods have passed beyond the stage where the cleaning up of backyards, or the penning up of pigs are considered matters of fundamental importance.

It is not well for a health officer to occupy himself too much with these matters. His legitimate work is much deeper and is more far-reaching in its effects. It is probable that we could have the hogs kept in their proper places, but after all such measures would result in raising a strong opposition which might interfere with the more important measures which we have in view.

We do not at present attempt to interfere to any considerable extent with the personal habits of the residents of these Islands in spite of the fact that some of these habits are very unsanitary, but are occupying ourselves with matters of greater importance. We are attempting to vaccinate the entire population against smallpox and are bringing about very gratifying results. In the provinces immediately adjacent to Manila the annual deaths from smallpox until very recently amounted to at least 6,000, but last year there was not a single death in these provinces from this disease.

We have been urging the boring of artesian wells, particularly in towns where the water supply has been found to be especially bad. We find that the people are glad to utilize the comparatively pure artesian well water for drinking purposes, instead of the contaminated water from shallow wells and open springs and streams which they formerly drank. We find, furthermore, that in a number of towns where artesian wells have been bored the death rate has fallen from 30 to 50 per cent, and I contend that this is a much more important reform than would be the putting of all the hogs in pens.

My attitude is that the people should be left alone so far as concerns matters of minor importance and that we should concentrate our efforts on the obtaining of large results from the really fundamental measures we now have under way. The people have now begun to be influenced by those of their number who are educated in hygiene. We do not need to go back farther than 1902 and 1903 in order to note a striking change. At that time the method universally employed in combating cholera was to hold religious processions, but we have patiently instructed the people in hygiene and since 1905 religious processions have been regarded as almost a negligible factor in preventing the spread of this disease. This result is one of very great importance. We should attempt to make effective measures which will reach and influence thousands rather than those which will reach tens.

Sir Allan Perry, Principal Civil Medical Officer, Ceylon: I wish to congratulate the gentleman on his paper. He naturally expects conditions to improve, and he regrets that after an absence of some years he comes back and finds them the same as when he left. My observations have been made in places that have been under the control of our government for one hundred years, and the conditions we encountered are very much the same there as they are here. It seems to me that he desires to go too fast. We can not alter the condition of the people simply by making laws. They will not observe them, but it is to be hoped that by education in modern sanitary measures they can be persuaded to bring about improvements of themselves. One more thing has struck me, and that is the influence of heredity on the condition of a people new to modern ideas.

Dean C. Worcester, Secretary of the Interior and Member of the Philippine Commission, Manila: There is one practical query which suggests itself in connection with Dr. Jackson's recommendation that we now proceed to carry out sanitary regulations by force. Can this be done in actual practice, or is the program which he outlines too ambitious?

A short time ago there was submitted to me a remarkable series of health ordinances intended to apply to the Igorots of the Province of Benguet. They were drawn up by an American health officer residing at Baguio who had obtained copies of the sanitary ordinances of the city of Manila and had persuaded the Igorot township council of Baguio to pass many of the most important of them and was attempting to enforce them among the wild people.

One must know these people in order fully to appreciate the absurdity of such an attempt. The ordinances contain specific provisions as to the construction and location of water-closets, when in point of fact no Igorot in Benguet Province has such a thing, if indeed there is any one of them who has ever heard of their existence. If an Igorot desired to dig a well it was made incumbent upon him to send to Manila and get the Director of Health to approve the site selected. It was necessary

for me to disapprove this whole series and to bring about the enactment in the form of ordinances of a few simple hygienic rules suited to the conditions and to the intelligence of these primitive people.

Referring to Dr. Jackson's statements relative to the opposition likely to be encountered in carrying out the program which he outlines, permit me to suggest that two kinds of opposition are certain to be encountered in this country, namely, active opposition and passive opposition. The first has not been lacking and Dr. Jackson has evidently never heard of certain facts which stick in my memory.

Not only have we had the efforts of efficient men rendered utterly futile by the active opposition of demagogues and politicians who have stirred up the people, but we have had sanitary inspectors murdered outright. While we are doubtless entirely capable with the armed force at our disposal of sitting on the lid no matter how much political fermentation the agitators may succeed in stimulating, it is, I am certain, far more easy to *persuade* people such as these with whom we are dealing in these Islands, to observe sanitary ordinances, than it is to compel them to do so, and disturbances of public order are not helpful if they can be avoided.

But however serious may be the results of active opposition the difficulties and obstacles created by the *passive* opposition of an Oriental people like the Filipinos are far more formidable. I am sure that every one of us who has encountered this opposition has an especially keen appreciation of Kipling's description of the sad fate of the man "who tried to hustle the East."

Dr. Jackson states that we have ordinances to cover everything, but that they are not enforced. I say that at the present time it is impossible to enforce all of them unless you pin them to the backs of the people with the bayonet, and to attempt to inaugurate such a policy would be foolhardy in the extreme.

Our general sanitary measures for the suppresion of smallpox, cholera and leprosy appeal strongly to the people. They can see and appreciate what we are doing along this line and we are gradually gaining their confidence and support.

While we have a large body of Filipino physicians in Manila who understand how to deal with individual cases of illness, these men have not had any general sanitary training. It was impossible for them to get it in Spanish times and we ought not to expect them to have it now. Until we have in these Islands a very much larger number of well-trained men who know the customs of the people and who can persuade them to adopt sanitary reforms without raising needless opposition, the fundamental laws of sanitation will continue to be violated. It takes *men* of the right sort to do these things, but suitable men are so scare at present that we can not fill our vacant positions. How many men would it take to carry out Dr. Jackson's program of enforcing sanitary

laws and ordinances by force in one such town as San Fernando, La Union, with its 64 separate barrios or villages?

We should not forget that even in the United States sanitary conditions which leave much to be desired are only too common. How then can we expect immediately to remedy such conditions here? It is so easy to pass a good law and then to imagine that all difficulties have been met and solved. It would, I am sure, be easy to secure a majority vote of the Philippine Legislature in favor of a law providing for the entry into the kingdom of heaven of all of the inhabitants of the Philippine Islands, but I fear that the passage of such an act would work no material change in the present condition of public morals.

In actual practice we find it necessary to advance little by little, feeling our way as we go. The attempt to enforce sanitary measures which are scientifically correct has not infrequently done serious harm in the past. The mistakes of this kind made during the cholera epidemic of 1902 are still green in the memories of many of us. We succeeded in arousing hostility and opposition which for some time went far toward nullifying our work. In drafting and enacting legislation we must seek to get measures which will bring results rather than those which look well on paper but can not be enforced in actual practice.

We are rapidly reducing the number of victims of leprosy and smallpox. We have succeeded in completely eliminating bubonic plague. We are confronted by the grave problems presented by the prevalence of tuberculosis and of hookworm disease. In my opinion we should concentrate our efforts for the present upon combating these diseases, which cause such a shocking mortality. After we have eliminated them we can turn our attention to ordinances dealing with less important matters. I hope for the time when a comprehensive series of health ordinances can be enforced throughout these Islands as I hope for the millennium, but it is my opinion that one is about as distant as the other.

Dr. Henry J. Nichols, first lieutenant, Medical Corps, United States Army: Dr. Jackson has had a considerable experience in the Philippine Islands. He has been on the ground and has personally seen many things. His views are not Quixotic, and I fully agree with him in certain of his suggestions. Manila has been made a beautiful spot, and those of us who have been in the provinces and have seen the lamentable lack of sanitary measures, find that a great discrepancy exists between Manila and the provinces, not only as relates to disregard of the law, but also to a disregard of personal habits relating to cleanliness.

Dr. N. M. Saleeby, superintendent of the University Hospital, Manila: There is left but little for me to say. I have had experience in the provinces. I served on provincial councils, and had a chance to look into these matters. I have seen small towns where a change of officials made marvellous improvement in the conditions, and I think the whole is a matter of local government. It will take a considerable time before

we can get these matters straightened out, but I want simply to emphasize the fact that much of what we call insanitary conditions in the provinces can be laid to the door of the municipalities.

Dr. Henry Fraser, director of the Institute for Medical Research, Kaula Lumpur, F. M. S.: The country which we have occupied for thirty years, The Federated Malay States, is healthful and attention is, of course, directed to the water supply, and not to the particular factors in life. The main work is done in the outlying districts.

As regards our efforts in sanitation, such as the combating of malaria, the government has expended large sums of money which are now bringing about good results. In reference to the education of the laity in combating disease, instructions are given in simple language to the school children and by them given to their parents. This method has proved satisfactory.

Dr. Jackson: I am sorry that my paper has been taken as a reflection on the health authorities and the Government. I believe in the Government of the Philippine Islands, but on the other hand I think the individual has a right to his views, and I only expressed views that I have observed in conversation with other Americans. My own personal view, which is all I put forward here, is that the native of the Philippines has a great respect for law, and I believe these laws as they now exist can be enforced without expense, and I believe with Dr. Saleeby that the trouble lies with the local authorities.

I do not hesitate to say that the conditions as they are now can, within a very few months, without the expenditure of very much money, be remedied and the ordinances relating to sanitation be enforced. I know the Filipino people well enough to know that if the leading men of the towns were instructed and told what to do, they would help us. If the enforcement of these laws could be placed upon the health officers of the provinces and at the expense of their positions, I think that the laws would be obeyed in six months' time. I do not lay much value on the sweeping up of towns, and in this respect I have nothing to offer to the Health Department or to the Government, but I think what we need is criticism. My criticisms in this matter are entirely friendly and I am sorry that anyone should take any other view of it.

Dean C. Worcester: I regret that Dr. Jackson should feel that anyone connected with the Bureau of Health would object to honest criticism. He certainly ought to know by this time that it has become impossible to hurt the feelings of Dr. Heiser or of myself. We have developed hides as thick as those of a pair of rhinoceri and the darts of the enemy produce not the slightest impression upon them.

But Dr. Jackson must not expect to make criticisms without drawing our fire in return. I understand this to be a fitting occasion for the free interchange of ideas. I spoke somewhat hastily, as I had a good deal to say and knew that Dr. Musgrave was holding the watch on me and

might call time at any moment and I perhaps failed to make my meaning entirely clear. I do not object to anything that Dr. Jackson has said. We are only too well aware of the fact that we have made mistakes in the past and are more than glad to have any well-meant suggestions as to how we may do better in the future, but in closing permit me to say that any one who talks of bringing about a general enforcement of sanitary regulations throughout these Islands within a period of a few months has a very imperfect conception of the problem with which we are confronted. We are doing all we can with the money and men at our disposal. Certainly many of the members of this association know that the death roll of Americans in these Islands bears the name of more than one faithful and efficient sanitary officer who has killed himself with overwork.

I insist that it will take a much larger body of men than we now have, as well as enormously increased appropriations for health purposes to bring about such conditions as Dr. Jackson believes could be readily secured and it should be borne in mind that I am not a pessimist on this subject.

I remember only too well that at the inauguration of our sanitary work here I was called insane because I believed that we could hope to accomplish certain things which have now been done, and I wish that it might prove true that Dr. Jackson is right and that I am wrong. The picture which Dr. Jackson has painted of conditions in our provinces and municipalities is absolutely true to life. The practical question is "what are we going to do about it"? We are not at all sensitive over having our past shortcomings criticised and what we *particularly* desire is a practical illustration of how it ought to be done. Nothing would afford me greater pleasure than to approve the appointment of Dr. Jackson as a district health officer and to afford him all possible support in an effort to realize in only a single health district his expectations as to what may be done.

REVIEW.

Clinical Bacteriology and Hæmatology for Practitioners. By W. D'Este Emery, M. D., B. Sc. Lond. Third edition. Cloth. Pp. xxxiv+252. Price $2 net. Philadelphia: P. Blakiston's Son & Co., 1908.

The author has endeavored to bring into the narrow confines of 250 pages those fundamental facts of bacteriology and hæmatology which are needed by a physician who does not have access to a good laboratory. The subject matter is presented in a remarkably clear manner and the arrangement is such that for any particular examination a description of the necessary apparatus and method of procedure is found in full, so that the reader does not have to refer to various portions of the book to obtain the desired information. This entails a certain amount of repetition, but greatly increases the practical value of the book.

Since bacteriology has been taught in almost all medical schools for a number of years past, the younger generation of physicians will find much here that would seem superfluous, along with many valuable suggestions as to the clinical applications of his bacteriological knowledge. The book is written, however, primarily for the older generation who did not have any bacteriological training and for them it will prove to be entirely satisfactory.

Only very few errors have crept into the work. One misses bile media in the blood culture of typhoid and V. Pirquet's cutaneous reaction for tuberculosis. Owing to the entirely doubtful value of opsonic index determinations, it would seem that too much space is given to their discussion.

The section on hæmotology is taken up mainly with the estimation of the amount of hæmoglobin, red and white cell counts, and the differential leucocyte count.

The accompanying plates are very satisfactory and add materially to the usefulness of the book.

The work as a whole is to be commended for its clearness and the attention that has been given to details of practical value to the practitioner.

OSCAR TEAGUE.